INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

A Xerox Education Company

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

73-4292

CAMERON III, Raymond Darwin, 1941-
WATER METABOLISM BY REINDEER (RANGIFER TARANDUS).

University of Alaska, Ph.D., 1972
Biology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

© 1972

Raymond Darwin Cameron III

ALL RIGHTS RESERVED

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

WATER METABOLISM BY REINDEER

(*RANGIFER TARANDUS*)

A

DISSERTATION

Presented to the Faculty of the

University of Alaska in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

by

Raymond D. Cameron III, B.S.

Fairbanks, Alaska

May 1972

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

WATER METABOLISM BY REINDEER

(RANGIFER TARANDUS)

APPROVED:

Chairman

APPROVED: _____ DATE: 11 May 1972

Vice President for Research
and Advanced Study

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

PLEASE NOTE:

Some pages may have

indistinct print.

Filmed as received.

University Microfilms, A Xerox Education Company

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

The effects of climatic and nutritional variation on body fluid compartmentalization and turnover were investigated in female reindeer. An initial field study characterized these changes on a seasonal basis under natural grazing conditions. Between early winter and late spring body weights were either maintained or reduced, while total body water (percentage of body weight) increased, indicating losses of body solids. During summer, body weight gains were accompanied by decreases in the percentage of body water, denoting an accumulation of body solids. Water flux rates were higher in late spring than during other seasons; lowest values were recorded in early winter.

A laboratory study was subsequently undertaken to ascertain the differential influences of temperature and nutrition on water flux. At low temperatures (-5 to -20°C) water flux is linearly related to nitrogen intake, and a direct relationship was found between the excretion rates of fecal water and nitrogen. At higher temperatures (+10°C) water flux increases relative to the intake of nitrogen due to higher rates of insensible water loss and an increase in the ratio of fecal water to fecal nitrogen excretion. Urine volume varies directly with water flux, independent of temperature and nitrogen intake.

Variations in nutritional status and changes in water turnover are discussed in relation to climate and the quality and availability of feed, and with regard to mechanisms for the conservation of energy and nitrogen.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGEMENTS

Fondest thanks are extended to Professor Jack R. Luick, friend and academic advisor, for nearly five years of skillful supervision. Without his continued support and sustained confidence, this work would have never reached a conclusion.

I am sincerely grateful to Dr. Robert G. White and Mr. Steve J. Person for numerous helpful suggestions in the design and execution of this study and for many hours of academic companionship.

Thanks are extended to Dr. Dan F. Holleman for invaluable assistance in radioisotope assay and statistical analysis, and to Mr. A. M. Gau and Miss Diane Carley for technical assistance.

I am indebted to Mrs. Betty Mather, Mrs. Peggy Woodlee, Mrs. Helga Wilm and especially to Mrs. Candy Anderson for the physical preparation of this volume. The skillful reproduction of figures by Mr. Don Borchert is gratefully acknowledged.

I thank Professor Peter R. Morrison, Director of the Institute, for continued financial support through numerous delays in the completion of this document.

Lastly, I wish to thank my wife, Jill, and my daughter, Amy, for their understanding and encouragement throughout the course of this investigation.

Financial support was provided jointly by the Atomic Energy Commission (Contract AT(04-3)-310), the National Institutes of Health (Contract 8-T01-OH-00031-03) and the Institute of Arctic Biology.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(TABLE OF CONTENTS, CONT.)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF ILLUSTRATIONS

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(LIST OF ILLUSTRATIONS, CONT.)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

INTRODUCTION

As a component of all cellular structures and a medium for all chemical reactions, water is the most critical nutrient. It is compatible with a variety of substances and is therefore an ideal medium for the transport of other nutrients. The high conductivity of water points to its importance in temperature regulation, both as a medium for even heat distribution within the body and as a vehicle for the removal of excess heat released by cellular metabolism.

The mammalian body contains more water than any other compound, the amount being nearly constant at 73% of the fat-free mass (Pace and Rathbun 1945; Panaretto 1963a), and because of the relative constancy of water in the fat-free mass, the percentages of fat and water in the whole body are inversely related. Further, since water has been associated with the maintenance of protein structure, the amounts of water and nitrogen tend to vary directly (Panaretto, 1963b). Thus, if the amount of water is known, the amounts of fat and protein may be estimated.

As a mammal grows, the size of the body water pool increases, but decreases as a percentage of body weight; such a change is associated with an increase in the ratio of less hydrated adipose, fibrous and collagen tissues to the more hydrated skeletal muscle (Kamal and Seif 1969a). Although both fat and protein accumulate during growth, the ratio of fat to protein gain is not constant in the young growing animal and the energy value of the gain varies as

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

a consequence (Searle and Graham 1970).

Several studies of the water metabolism of ungulates in pathological states appear in the recent literature and include the effects of dehydration (Bullard et al. 1970; Maloiy 1970; Macfarlane et al. 1961), parasite burdens (Baker et al. 1965) and diarrhetic conditions (Phillips and Knox 1969). Other investigations have been concerned with defining the adaptive responses of ruminants to arid heat, and such findings have been reviewed by Macfarlane (1964) and Schmidt-Nielsen (1964). On the other hand, water metabolism of arctic species has not been widely investigated. The responses of reindeer to heat stress and water deprivation have been studied (Rosenmann and Morrison 1967), but no comprehensive study of water metabolism in reindeer or caribou has ever been undertaken.

The overall objective of this study was to determine the influence of seasonal changes in climatic and nutritional factors on the compartmentalization and turnover of body water in reindeer, and consists of two separate but closely related phases. The objective of the first phase (Chapter 1) was to characterize seasonal changes in total body water volume and turnover, extracellular fluid volume, and blood volume of reindeer under natural grazing conditions. The results of this field work stimulated a laboratory investigation (Chapter 2) which was designed primarily to assess the differential effects of climatic and nutritional variations on water flux in this species. This work is intended as a contribution to the growing volume of information available on the adaptive responses of mammals

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to cold environments and is concerned with a herbivore which constitutes a significant part of arctic and subarctic ecosystems.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 1

SEASONAL CHANGES IN TOTAL BODY WATER, EXTRACELLULAR FLUID, AND BLOOD VOLUME IN GRAZING REINDEER

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

INTRODUCTION

Reindeer and caribou, Rangifer tarandus, inhabit the tundra and taiga of the arctic and subarctic and are subjected to climatic and nutritional circumstances of marked seasonal variation. The winter diet consists chiefly of lichen, and species typical of those consumed are low in crude protein, averaging less than 3% (Kelsall 1968). Reindeer and caribou enter the spring season in poor body condition which in pregnant females is exacerbated by increased fetal requirements near parturition and by the metabolic demands of lactation. The availability of food at this time, as controlled by the depth and encrustation of snow, may thus be a critical factor in reproductive performance. The onset of summer marks a dietary shift from lichen to grasses, sedges and deciduous vegetation of higher nutritive value which presumably accounts for the deposition of large fat stores throughout the summer and early fall. Body weight increases and general improvement of body condition during this period are assumed important for subsequent winter survival.

This report describes seasonal changes in total body water volume and turnover, extracellular fluid volume, and blood volume of grazing female reindeer using tritium water, radiosulfate-^{35}S and radiochromate-^{51}Cr, respectively. A study of these parameters under the contrasting seasonal circumstances encountered by Rangifer is important in defining changes in body composition as influenced by differing nutritional circumstances, and should provide an insight

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

into the adaptive responses of the species to environmental extremes. The estimation of total body water volume is particularly useful in studies of body composition since it permits body weight change to be partitioned into solid and aqueous component changes. In addition, the extracellular and intravascular compartments as well as the turnover of body water may be responsive to acute changes in climate or food supply.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

MATERIALS AND METHODS

Animals

Seven female reindeer ranging in age from 1.5 to 4 years were used in this study. Experiments were conducted with non-pregnant cows from December 1968 through August 1969; in subsequent trials all cows were pregnant or lactating with two exceptions: No. 9 failed to conceive and No. 14 gave birth to a stillborn calf at term. Otherwise, the cows calved normally and lactated through the final trial in June 1970.

Design

The experiments were conducted at the University's Reindeer Research Station near Cantwell, Alaska, where the deer were confined to paddocks and allowed to forage the native vegetation. A detailed description of these paddocks, including vegetative composition appears elsewhere (Luick et al. 1971). On two occasions (Janaury--February 1969 and April--May 1970) supplemental feed (Cattle Starter #2, Ralston-Purina Corp., St. Louis, Mo.) was provided to prevent severe depletion of lichen pastures and to insure survival of the animals. Supplementation was discontinued at least 3 weeks prior to the commencement of field experiments.

The reindeer were herded into a holding corral and restrained in stalls for isotope injections and/or withdrawal of blood samples. All injections were made within 3 hours of removal from pasture, and animals were again released at the conclusion of each day's collection

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

period. All injections and blood samples were by jugular venipuncture; sodium heparin was used routinely as an anticoagulant.

Radioisotope Injections and Sampling Protocol

Tritiated water and sodium sulfate-^{35}S (New England Nuclear Corp., Boston, Mass.) were diluted with 0.9% (w/v) sterile saline to specific activities of 1 mCi/ml and 40 uCi/ml, respectively. A 10 ml pre-injection blood sample was obtained, tritiated water (3 mCi) was injected intravenously and blood samples were taken at regular intervals during the following 2 to 3-week period. Sodium sulfate-^{35}S (200uCi) was also injected intravenously following pre-injection sampling, and blood samples were withdrawn at 30, 60, 90, 120, 150, and 180 min post-injection.

The procedure used for labeling red blood cells with ^{51}Cr was similar to that described elsewhere (Hoye 1967; Stahl and Dale 1958). Jugular blood (8 ml) was drawn into a syringe containing 2 ml of Special Formula A-C-D Solution (Abbott Laboratories, North Chicago, Ill.) and transferred to a test tube. Approximately 20 uCi of sodium chromate-^{51}Cr (Abbott Laboratories, North Chicago, Ill.) was added and the mixture was incubated for 30 minutes at room temperature (20-30°C) with intermittent swirling. After incubation, 50 mg of sterile ascorbic acid was added, the blood was centrifuged, the plasma removed and the red cell fraction was resuspended in sterile saline to the original volume. Centrifugation and resuspension were repeated twice after which a 3 ml aliquot was removed for radioassay. A 10 ml pre-injection sample was drawn

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

from the animal and a 5 ml portion of the remaining suspension was injected intravenously. One 12 ml blood sample was taken 15 min post-injection; microhematocrit determinations were made in duplicate.

Analytical Procedures

Water was recovered from plasma samples by vacuum sublimation, and a 1 ml aliquot of each plasma water was added to 10 ml of scintillation solution consisting of a 4 to 9 (v/v) mixture of toluene and Triton X-100 (Rohm and Haas, Philadelphia, Pa.) with 4 g/l of 2,5-diphenyloxazole (PPO) and 100mg/l of 1,4-bis [2-(5-phenyloxazolyl)-benzene] (POPOP). Standards were prepared in a similar manner from the original injection solution diluted $1:10^5$. Plasma samples from radiosulfate trials and an aliquot of the diluted injection solution ($1:10^3$) were prepared for radioassay by the method of Jeffay et al. (1960). A Nuclear Chicago Mark I liquid scintillation counting system was used for radioassay of tritium and ^{35}S. Quenching was evaluated by the channels ratio method. Duplicate 5 ml aliquots of whole blood and diluted samples of injected ^{51}Cr-RBC's were hemolyzed to insure uniform counting geometry and assayed in a Nuclear Chicago "TOBOR" detector (NaI crystal) and shielding assembly with an associated dual channel pulse height analyzer and scaler.

Calculations

Total Body Water Volume (TOH Space) and Water Turnover

The decrease in plasma TOH activity with time is described by a single exponential expression: $\alpha_t = \alpha_o e^{-kt}$, where α_t is the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

specific activity (cpm/ml) at any time t(days) after injection of the tracer. The specific activity of body water at time of injection (α_o) is approximated by extrapolation of the exponential regression line to zero time. The daily fractional turnover rate of body water (k) equals the slope of the regression line. Total body water (TBW) was calculated from the relation: TBW (ml) = $\frac{\beta}{\alpha_o - \alpha_{t'}}$. β is the dose injected (cpm) and α_t, is the specific activity (cpm/ml) of the pre-injection sample. The biological halftime ($t_{1/2}$) is the time required for the body to eliminate one half of an injected dose of TOH and is determined by: $t_{1/2}$ (days) = 0.693/k. The water flux rate (a) expresses the amount of water entering or leaving the body water pool per unit time and is calculated from the equation: a(ml/day) = k • TBW.

Extracellular Fluid Volume (Radiosulfate Space)

As with TOH, intravenously injected radiosulfate is eliminated from the plasma at an expontial rate after mixing is complete. Extracellular fluid volume (ECF) is determined from: ECF (ml) = $\frac{\beta}{\alpha_o - \alpha_{t'}}(0.93)(0.90)$ where β is the injected dose (cpm), α_o and $\alpha_{t'}$ are the zero time and pre-injection specific activities (cpm/ml plasma), respectively; 0.93 corrects for the water content of plasma and 0.90 is the Donnan factor for sulfate (Walser et al. 1953).

Blood Volume

Total blood volume (BV) was calculated from the equation: BV (ml) = $\frac{\beta}{\alpha_{15}}$; where α_{15} is the specific activity (cpm/ml whole blood) for the sample drawn at 15 minutes post-injection. Red cell volume

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(RCV) was estimated by: RCV (ml) - (BV)(H)(0.97), where H is the fractional hematocrit and the factor 0.97 corrects for trapped plasma (Hodgetts et al. 1959). Plasma volume (PV) was estimated by the difference between total blood volume and red cell volume.

Statistical Methods

The method of least squares was used for regression analysis of exponential relationships. Standard errors of means and levels of significance were calculated by standard methods.

Climatological Data

Climatological records for Summit, Alaska were obtained from the U. S. Department of Commerce, Environmental Science Services Administration. Summit is located 16 km south of the Reindeer Research Station; the two areas are subject to very similar conditions of temperature and precipitation. Table I lists mean climatological data for each of the seven experimental periods.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE I

Mean climatological data* for the duration of each experimental period**

Month	Temperature, °C	Wind velocity, m/sec	Cumulative snow cover, cm
December 1968	-21	8.3	10
March 1969	-9	4.2	33
May 1969	+2	4.2	5
August 1969	+7	3.6	0
December 1969	-11	5.8	58
March 1970	-6	4.6	79
June 1970	+9	4.8	0

* Summaries of local climatological records for Summit, Alaska obtained from the U. S. Department of Commerce, Environmental Science Services Administration.

**For complete tabulation showing annual trends see Luick et al., 1971.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

RESULTS

Values obtained for total body water volume, water flux, extracellular fluid volume and the intravascular volumes are presented as a percentage of body weight (Table 2), and appear diagrammatically in liters based on the mean body weight for each experimental period (Figure I). In general, total body water as a percent of body weight was lowest in late summer, increased in winter, and was highest in late spring. The period from December 1968 to May 1969, when reindeer were non-pregnant, was characterized by a significant increase ($p<0.05$) in total body water, but with negligible net change in body weight indicating an absolute reduction in total body solids. Despite this progressive expansion in total water content and the five-fold increase ($p<0.05$) in water flux rate, mean extracellular fluid volume was significantly lower ($p<0.05$) in March than in December; changes in total blood volume were not significant. The large body weight gain between May and August of 1969 was accompanied by a decrease ($p<0.01$) in total body water denoting a considerable gain in total body solids. Water flux decreased ($p<0.01$) to a value approximating that obtained for the preceding March. Between August and December 1969 body weights rose only slightly while total body water increased significantly ($p<0.01$) demonstrating again a reduction in total body solids; changes in water flux were not significant. During the period from December 1969 to June 1970, total body water

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

remained relatively unchanged as body weights progressively decreased; but except for one case, individual body weight losses exceeded the corresponding reductions in total body water indicating, as in the previous winter, a net loss in total body solids. Water flux for this period increased ($p<0.01$) threefold, and significant changes in other compartment volumes were apparent: mean extracellular fluid volume increased ($p<0.01$) whereas, total blood, red cell, and plasma volumes decreased ($p<0.01$, $p<0.01$ and $p<0.05$, respectively.)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 2

Seasonal changes in total body water volume, water turnover, extracellular fluid volume, and blood volume in mature female reindeer

Month	Reindeer No.	Body weight, kg	Total body water vol., %	$t_{1/2}$, days	Water flux rate, (ml/day)/kg	Extra-cellular fluid vol., %	Total blood vol., %	Hema-tocrit, %	Red cell vol., %	Plasma vol., %
December 1968	5	72.0	71.1	14.7	34	17.5	8.9	43.0	3.7	5.2
	9	80.5	63.1	16.5	27	19.0	8.3	43.0	3.5	4.8
	10	88.0	60.1	16.5	25	16.4	9.8	52.0	4.9	4.9
Mean ±S.E.*			64.8 ±3.3	15.9 ±0.6	29 ±3	17.6 ±0.8	9.0 ±0.4	46.0 ±3.0	4.0 ±0.4	4.9 ±0.1
March 1969	5	78.0	76.3	7.7	69	10.5				
	9	87.0	68.4	7.8	61	8.9				
	10	93.0	69.2	9.1	53	9.8				
Mean ±S.E.*			71.3 ±2.5	8.2 ±0.5	61 ±5	9.7 ±0.5				
May 1969	5	72.5	82.1	5.0	114		8.6			
	9	80.5		3.8			7.1			
	10	87.0	78.7	3.1	176		10.6			
Mean ±S.E.			80.4 ±1.7	4.0 ±0.6	145 ±31		8.8 ±1.0			

Month	Reindeer No.	Body weight kg	Total body water vol., %	$t_{1/2}$, days	Water flux rate, (ml/day)/kg	Extra-cellular fluid vol., %	Total blood vol., %	Hema-tocrit, %	Red cell vol., %	Plasma vol., %
August 1969	2	89.0	53.6	7.2	52					
	5	96.5	51.3	7.4	48					
	9	103.5	51.5	6.9	52					
	10	111.5	44.0	5.3	58					
	14	81.5	69.3	5.8	83					
	17	96.5	53.7	7.4	50					
Mean ±S.E.*			53.9 ±3.4	6.7 ±0.4	57 ±5					
December 1969	2	92.0	64.1	7.9	56					
	5	103.0	71.5	8.6	58	12.5	8.2	49.0	3.9	4.3
	9	107.5	69.7	9.5	51	12.9	8.0	41.5	3.2	4.8
	10	115.5	75.8	9.9	53	10.8	7.4	48.0	3.4	4.0
	12	111.0	72.3	7.5	67	13.8				
	14	83.5	76.0	6.8	78	15.0				
	17	102.5	70.0	7.8	62	14.0				
Mean ±S.E.			71.3 ±1.5	8.3 ±0.4	61 ±4	13.2 ±0.6	7.9 ±0.2	46.2 ±2.4	3.5 ±0.2	4.4 ±0.2

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Month	Reindeer No.	Body weight, kg	Total body water vol., %	$t_{1/2}$, days	Water flux rate, (ml/day)/kg	Extra-cellular fluid vol., %	Total blood vol., %	Hema-tocrit, %	Red cell vol., %	Plasma vol., %
March 1970	5	96.5	62.3	5.0	86	13.8	8.2	48.0	3.8	4.4
	9	97.5	70.9	7.9	62	11.4	8.3	44.0	3.5	4.8
	10	112.0	72.5	9.5	53	10.3	6.5	45.0	2.8	3.7
	12	103.0	68.7	6.7	71	12.1	6.1	42.0	2.5	3.6
	14	79.5	71.3	5.4	92	15.7	7.1	43.0	3.0	4.1
Mean			69.7	6.8	75	12.5	7.0	44.2	3.0	4.0
±S.E.			±1.6	±0.7	±6	±0.8	±0.5	±0.9	±0.2	±0.2
June 1970	2	76.0	70.1	2.5	194					
	5	77.5	79.2	2.7	203	16.6	6.4	41.0	2.5	3.9
	9	88.5	55.0	2.7	141	17.5	4.5	44.0	1.9	2.6
	10	86.0	79.0	2.9	189	20.6	4.2	43.0	1.8	2.4
	12	86.0	82.3	2.7	211	20.4	6.0	35.0	2.0	4.0
	14	68.0	79.1	3.1	177	19.4	4.6	39.0	1.7	2.9
	17	76.5	80.1	2.8	198	18.9	4.7	40.5	1.8	2.9
Mean			75.0	2.8	188	18.9	5.1	40.4	2.0	3.1
±S.E.			±3.6	±0.1	±9	±0.6	±0.4	±1.3	±0.1	±0.3

* Standard error of the mean.

NOTE: Volumes are expressed as a percentage of body weight.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

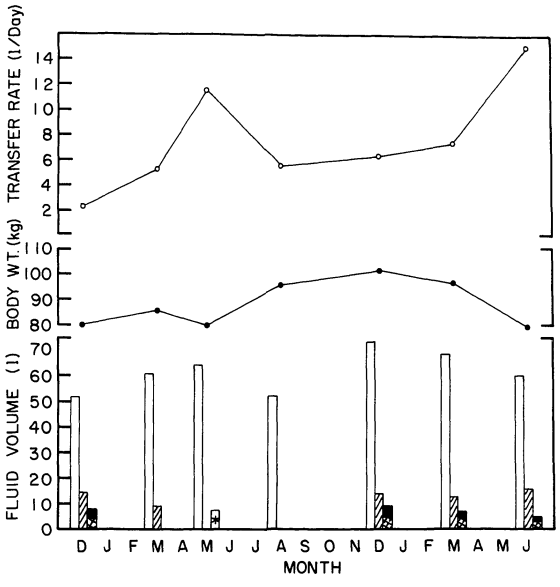


FIGURE 1. Diagrammatic representation of mean seasonal trends of water flux rate (o), total body water volume (□), extracellular fluid volume (hatched bar), red cell volume (■), and plasma volume (cross-hatched bar) in female reindeer on the basis of mean body weight (●). * = no hematocrits taken; only total volume shown.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DISCUSSION

In recent years, the tritiated water dilution method has been used extensively for the in vivo estimation of total body water and related kinetic parameters. However, injected tritium exchanges with hydrogen in organic body constituents with the result that the apparent volume of distribution of TOH increases. Pinson (1952) calculated the water equivalent for the readily exchangeable hydrogen atoms of the animal body to be on the order of 1-2%, and it has been recommended that TOH spaces be reduced by 2% to correct for this exchange (Prentice et al. 1952). In the present study, uncorrected TOH space is equated with total body water volume, the primary purpose being to ascertain relative changes in body water parameters with respect to season, the determination of absolute volumes being of secondary importance.

Radiosulfate-^{35}S has been used here to estimate the volume of the functional extracellular fluid which is defined by Walser et al. (1953) as "that portion of chloride-containing body fluid which is responsive to acute changes in the composition of the plasma." These authors report a greater rate of urinary loss of injected radiosulfate during equilibration (4-8% of the injected dose) than during the period following equilibration in humans and dogs; and the specific activity of serum radiosulfate at equilibrium (15-30 min post-injection) was therefore increased by a constant value to obtain the theoretical specific activity at

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the time of injection, from which the volume of distribution was calculated. In the present study values for the radiosulfate space were calculated from uncorrected zero time specific activities obtained by extrapolation of the exponential regression and therefore may be slightly in excess of true volumes. Again, since this study is essentially of a comparative nature, observed changes in the radiosulfate space are assumed to represent differences in the volume of the functional extracellular fluid.

The ^{51}Cr-tagging technique described above provides an accurate estimate of circulating blood volume only if jugular hematocrit is representative of the whole body. The ratio of cells to plasma however, is not uniform throughout the vascular system, blood in the small vessels of the body having a significantly lower hematocrit than the large vessels (Schalm 1965). A further complication is the variations in splenic output of high-hematocrit blood associated with changes in emotional state (Turner and Hodgetts 1959). Hodgetts (1961) developed a factor, designated $F_{\underline{cells}}$, which corrects jugular hematocrit to whole body hematocrit in adrenalin-treated sheep. Of the data presented here (Table 2), total blood volumes are theoretically lower than true values due to the probability that ^{51}Cr specific activity of jugular blood taken at 15 min post-injection is higher than that of blood with a hematocrit representative of the whole body, and differs from the latter by the $F_{\underline{cells}}$ factor; thus, the volume of distribution of tagged red cells (calculated as total blood volume) is smaller than the true

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

volume. The red cell volume however, should equal the true volume since in the appropriate calculations the effect of an erroneous total blood volume is cancelled by the use of the proportionately higher jugular hematocrit. Plasma volumes, because they are determined by difference, are in error by the same amount as total blood volume. Subjectively, reindeer were calm during the experimental procedure, being accustomed to handling and restraint; since adrenalin was not administered and because mixing of tagged red cells with splenic blood reserves is presumably minimal during the 15-min equilibration period the results presented for the intravascular volumes, although subject to the inaccuracies outlined above, are presumed to reflect circulating levels and the seasonal changes thereof.

Changes in Body Fluid Compartments

It is well established that body water and body fat are inversely related, and it is therefore highly probable that the seasonal variations in total body water observed in reindeer are primarily a result of changes in body fat reserves which reflect contrasting nutritional circumstances. Springell (1968a) noted that high total body water values in grazing steers are associated with poor diet, and remain at high levels regardless of season, whereas in well-fed yarded steers total body water varies within a lower, seasonally-controlled range. In addition, seasonal changes in total body water of both domestic sheep and Columbian black-tailed deer under pen-fed conditions have been attributed primarily to differences in the amounts of stored fat (Longhurst et al. 1970).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Pace and Rathbun (1945) proposed the following formula for the determination of body fat: % Fat = 100 - $\frac{\% \text{ Water}}{0.732}$, where 0.732 is the mean proportion of water in the lean body mass for several mammalian species. This relationship assumes that the lean body mass contains all of the body water in a constant amount. The principal criticism of this concept is that lean body mass cannot be assumed to be of constant water content in pathological states (Prentice et al. 1952) or in situations of climatic or nutritional stress. For example, the large reductions in intracellular fluid volume accompanying acute dehydration in burros (Yousef et al. 1970) would almost certainly decrease the size of the aqueous component of the lean body.

Direct postmortem measurement of body fat following in vivo determination of total body water in sheep and goats has permitted the formulation of prediction equations for the indirect estimation of fat content in these species (Panaretto 1963a). However, there is no evidence to indicate that a prediction equation developed for one species may be accurately applied to another, nor is an environmentally stressed member of the same species likely to exhibit a fat-water interrelationship similar to that of a non-stressed individual. Thus, the application of the equations of both Pace and Rathbun (1945) and Panaretto (1963a) to total body water data from the present study results in mean negative fat values for May 1969 and June 1970. Intravenously administered tritiated water equilibrates with rumen water in 3-4 hours in cattle (Aschbacher et al. 1965), and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

therefore its volume of distribution includes this pool. Abnormal increases in alimentary water could therefore account for the higher total body water volumes and thus the erroneously low calculated values for percent body fat. Obviously, the accurate determination of body fat in reindeer by in vivo methods requires the formulation of prediction equations based on the relationship between carcass analysis and in vivo determinations over a wide range of nutritional circumstances.

Determinations of total body water in Friesian cattle in the Sahara climate (Kamal and Seif 1969b) demonstrate that high environmental temperatures induce a voluntary reduction in feed intake, an increase in total body water and a reduction in dry body weight (i.e., total body solids). This response is similar to results of the present study from December 1968 to May 1969. Extracellular fluid was reduced significantly from December 1968 to March 1969 (no values are available for May 1969) indicating an apparent increase in the intracellular component of the total body water pool (Figure 1). Since body fat consumed in the starved or undernourished state is apparently replaced by water in the adipose tissue (Farrell 1970), this suggests a major fluid shift from the extracellular to the intracellular compartment as a result of fat mobilization.

Although, aside from glycogen, fat is the most labile and concentrated of body energy reserves, there is apparently a concomitant utilization of body protein during periods of undernutrition (Farrell 1970). A mobilization of protein reserves may, in part,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reflect a demand for endogenous glucose precursors when exogenous supplied are low, although it is likely that some 3-carbon units are supplied by the glycerol moiety of mobilized triglycerides. An increased requirement for glucose would be expected during pregnanacy for growth and metabolism of fetal tissues and suggests a considerable utilization of body protein by undernourished pregnant reindeer. Furthermore, the delay in the attainment of peak body condition in summer may be regarded as a consequence of increased fetal demands in late pregnancy and the metabolic cost of lactation subsequent to parturition. In this regard it is of interest that the total body water value for the only non-pregnant, non-lactating cow (No. 9) is more than 25% lower than the mean value for lactating animals in June 1970 (Table 2), and its deletion from statistical calculations increases the June mean to 78.3% which is significantly higher than both December 1969 ($p<0.05$) and March 1970 ($p<0.01$) means. This supposition of body protein utilization gains support from the work of Panaretto (1964) who reported parallel decreases in body water and protein in ewes subjected to progressive undernutrition, and is further substantiated by the probably -- but as yet unestablished -- assumption that non-supplemented reindeer wintering on lichen range are in negative nitrogen balance.

Low protein intake associated with depletion of protein reserves, may also explain the increase in extracellular fluid volume and accompanying decrease in plasma volume observed between December 1969 and June 1970. Parasitic gastroenteritis in cattle is accompanied

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

by clinical symptoms of dehydration, emaciation, and interstitial edema (Baker et al. 1965). Apparently the accompanying reduction in feed intake induces a decrease in plasma protein and electrolyte concentrations and a consequent decrease in osmotic pressure, resulting in a net shift of plasma water to the interstitial spaces. Tissue hypertension has also been postulated as an explanation for total body water increases in heat stressed cattle (Kamal and Seif 1969b; Siebert and Macfarlane 1969) and water buffalo (Kamal and Seif 1969b) as a consequence of an increased water intake and an accompanying reduction in feed intake. It is likely that during June 1970 reindeer consumed forage of high moisture content which precipitated an interstitial edema by supplying water in excess of the requirement without providing sufficient energy and nitrogen for maintenance. The fact that water flux rates during this period were the highest recorded (Table I) tends to support this hypothesis.

The apparent deterioration of body condition between December 1969 and June 1970 was accompanied by decreases in total blood, red cell, and plasma volumes. Springell (1968b) obtained high correlations between the volumes of these vascular compartments and fasting body weight in steers which were subjected to variations in feed quality; increased hematocrits, red cell volumes and total blood volumes were generally associated with higher planes of nutrition although a clear pattern of seasonal changes was not obtained. In sheep red cell volume was found to vary with the logarithm of total body water, the latter serving as an index of lean mass (Panaretto

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and Little 1965). A correlation between red cell mass and lean body mass is sound in principle since a relationship almost certainly exists between the oxygen demand of the active body tissues and the red cell mass which functions in oxygen transport. Since the metabolic rate of adipose tissue is apparently very low (Doornenbal et al. 1962), red cell volume should reflect the amount of fat-free, or lean mass of the body. In fact Doornenbal et al. (1962) reported a linear relationship between ^{51}Cr-determined red cell volume and lean body mass in rats. Springell (1968b) however, states that the "prediction of lean body mass from the red cell content would be contingent on the constancy of a number of other variables and could be useful only in limited circumstances." Although we have been unable to establish a statistically significant relationship between red cell volume and total body water, the observed reduction in red cell volume and the calculated decrease in intracellular fluid volume between December 1969 and June 1970 (Figure 1) may serve as qualitative evidence that lean body mass was declining during this period.

Seasonal Changes in Water Turnover

Results indicate that water turnover in reindeer is markedly influenced by climatic and/or nutritional circumstances. Thus, the data in Table 2 show that flux rates are higher in late spring and early summer than in late summer, winter and early spring, and may reach extremely low levels in early winter as shown for December 1968. Similar observations of seasonality in water turnover

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

also been reported for deer and sheep (Longhurst et al. 1970) and for cattle (Siebert and Macfarlane 1969; Springell 1968a).

The present study shows that biological half-time ($t_{1/2}$) of TOH in the total body water pool tends to decrease with increasing ambient temperature (Figure 2). This suggests a direct influence of temperature on water turnover. Thus, in summer a higher water flux may be in part a consequence of increased evaporative losses. In winter when liquid water is unavailable, a reduced water intake would be valuable from the standpoint of energy conservation due to the metabolic cost of melting frozen water and raising it to body temperature. However, seasonal temperature variations are accompanied by changes in forage quality and availability which tend to confound the apparent, and possibly fortuitous, influence of temperature on water turnover. The availability of forage as controlled by range condition and snow cover (Table I) may exert a pronounced effect on water turnover. Forbes (1968) established a positive correlation between total water intake and dry matter intake in non-pregnant ewes for each of three different feeds. This author suggested that parallel increases in dry matter and water intakes may be explained by an increase in fecal water loss and by a higher urine production due to an increased metabolic rate at higher dry matter intakes. Similarly, Siebert and Macfarlane (1969) noted a higher water turnover in cattle with an increase in food supply. Aside from the more obvious changes in dry matter intake associated with the availability of forage, reindeer may

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

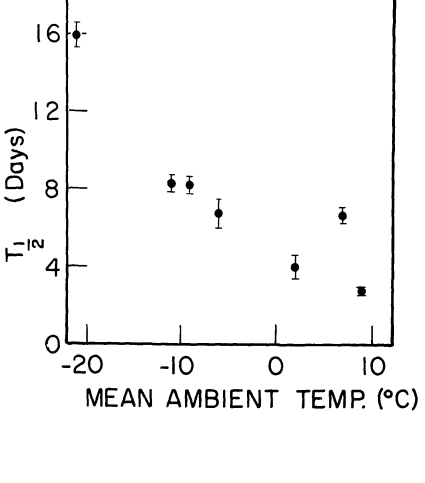


FIGURE 2. Biological half-time ($t_{1/2}$) plotted as a function of mean ambient temperature.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

voluntarily restrict their feed intakes in winter; McEwan (1968) has shown that captive caribou fed a pelleted ration ad libitum exhibit a cyclical growth pattern, consisting of a rapid body weight gain in the summer followed by a reduction and subsequent stasis in body weight during winter. The onset of estrus also marks a reduction in voluntary feed intake (McEwan 1968) and may account for the deviation shown in the $t_{1/2}$-temperature relationship for August 1969 (Figure 2) if accompanied by a decrease in water intake.

High water flux rates in May 1969 and in June 1970 may be a consequence of the high water content of forage resulting in a water intake in excess of the requirement and perhaps an increase in the alimentary component of the total body water pool (see previous section). Thus, between March and May 1969 increasing ambient temperatures reduced the snow cover from 33 to 5 cm (Table I) and the resulting runoff may have been absorbed by the forage to the point of near-saturation. Similarly, an unseasonable snowfall in early June 1970 added to the already substantial ground water from the normal spring thaw, again hydrating the low-lying forage. This, in addition to the probable high water content of young, standing browse may account for the high water flux rates observed in May 1969 and June 1970 (145 and 188 ml/day/kg, respectively). Macfarlane et al. (1966) have reported a daily water turnover of about 130 ml/day/kg in sheep grazing pastures in which water content of vegetation was in excess of 83%.

Seasonal differences in the protein content of forage available

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to reindeer may also have a significant effect on water flux. Increases in water turnover with the consumption of higher protein feeds have been reported for both grazing (Siebert and Macfarlane 1969) and yarded cattle (Springell 1968a). Likewise, Forbes (1968) has shown that sheep fed various amounts of cubed grass have higher total water intakes per unit of dry matter than those consuming hay at equal levels of dry matter, and suggested that the effect is due to the higher protein content of cubed grass. An increased water requirement at higher levels of protein intake could be a consequence of an increased production of nitrogenous wastes resulting in higher urinary water losses. Conversely, water restriction on a low nitrogen diet tends to reduce urinary losses of urea in sheep (Goodall and Kay 1968; Maloiy et al. 1970), red deer (Maloiy et al. 1970) and cattle (Livingston et al. 1962). The urea conserved is thought to cycle to the rumen and enhance microbial protein synthesis (Schmidt-Nielson et al. 1957a). Thus a reduced water intake by reindeer in winter not only assists in heat conservation but may also effect a more efficient utilization of nitrogen.

Paralleling the seasonality in protein content of forage are probably changes in the availability of mineral nutrients. In summer the minerals required for antler and bone growth and for milk production are apparently supplied in the forage and as dissolved salts in streams and potholes. In contrast, although the mineral requirement in winter is presumably somewhat lower, the ash content of lichen is low compared with that of plant species typically

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

selected in summer (Kelsall 1968; Klein 1968; Luick unpub.) and observations of the comsumption of antlers, bones, sea water, urine, and particulate matter by caribou in winter (Kelsall 1968) are highly suggestive of a mineral deficiency. The consumption of snow, which is essentially devoid of salts, would tend to compound an existing mineral deficit. It has been shown that water consumption is directly related to salt intake in sheep (Macfarlane et al. 1967; Wilson 1966) and it is therefore possible that water turnover in reindeer is also controlled by seasonal changes in the availability of major dietary electrolytes.

Pregnancy and lactation may account for the higher water flux rates observed in the spring and early summer months. Forbes (1968) reported that water intake per unit of dry matter is significantly higher in domestic female sheep from the fourteenth week of pregnancy through the fourth week of lactation than that of non-pregnant, non-lactating controls. In the present study however, mean water flux in March 1969, when reindeer were not pregnant, is not significantly different from the corresponding value in March 1970 (Table 2), when all except one (No. 9) were pregnant. Apparently if water flux was stimulated appreciably by pregnancy, the increase occurred close to parturition. Water flux rates for lactating reindeer were somewhat higher than those for the two non-lactating reindeer (Nos. 9 and 14) in June 1970 (Table 2). This is undoubtedly due in part to increased water losses via milk and as a result of the higher metabolic rates accompanying lactation.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Aschbacher et al. (1965) noted substantially shorter biological half-times ($t_{1/2}$) for lactating than for non-lactating dairy cattle although Black et al. (1964) reported no significant effects of lactation on this parameter. These latter workers hypothesized that lactation induces an expansion of the total body water pool with an accompanying increase in water flux such that $t_{1/2}$ is maintained at the non-lactating level. Thus, it is of interest to note that whereas $t_{1/2}$ values for No. 9 (non-lactating) and No. 14 (non-lactating but formerly pregnant) are similar to those for lactating reindeer, marked differences were found for water flux and (or) total body water volume (Table 2). These data confirm the hypothesis put forth by Black et al. (1964) and suggest further that the combined effects of late pregnancy and dietary insufficiency are relatively more important than lactation for inducing changes in body water kinetics.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHAPTER 2

EFFECT OF DIET AND TEMPERATURE ON KINETICS OF BODY WATER

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

INTRODUCTION

The previous study demonstrated that grazing reindeer exhibit a distinct seasonality in water flux. Reported values ranged from 29 ml/day/kg body weight in early winter to 188 ml/day/kg body weight in late spring. Because of the rapid melting of snow in late spring it seems likely that lichens and other low-lying vegetation would become saturated; the consumption of such wet forages and of young succulent plants may largely account for the extremely high rates of water flux observed in the spring season.

Changing weather conditions in the far north cause marked seasonal fluctuations not only in forage quality (Klein, 1970), but also, because of changing snow conditions, in the amounts of forage available to grazing reindeer. Both feed quality and dry matter intake are known to influence water turnover in other ruminants and it appears likely that these factors are of similar importance in reindeer.

Extremes of climate may also influence water flux of reindeer in a more direct manner. Winter temperatures in the arctic and subarctic may fall below -40°C, and since snow and ice are the only available sources of water, decreases in water intake may be necessary to conserve body heat. In contrast, higher summer temperatures may increase insensible losses of water, thereby increasing the water requirement.

The primary purpose of this study was to examine the differential effects of dry matter intake, protein intake, and ambient temperature

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

on water flux in reindeer as determined by the tritium water (TOH) dilution method. Also of major interest was an evaluation of the changes in body composition, nitrogen balance, and electrolyte status which accompanied various shifts in diet or temperature. The results are interpreted with consideration for changes in plasma constituents related to water, nitrogen and electrolyte metabolism. Lastly, the accuracy of measuring water flux using the TOH dilution method was tested by comparing results so obtained with direct measurements of water input.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

MATERIALS AND METHODS

Animals and Holding Facilities

Two non-pregnant female reindeer, ages 2.5 (No. 24) and 4.5 (No. 9) years, were used in this study. The animals had been held previously in a large outdoor paddock for several months and given an ad libitium diet of a commercial pelleted feed (Cattle Starter #1, Ralston-Purina Corp., St. Louis, Mo.). They were removed from the field enclosure in mid-October when peak body condition is normally attained, and placed in a 4.5 x 3.0 m controlled environmental chamber, each in a 2.5 x 1.5 m box stall. Although previously accustomed to handling and familiar with experimental routines in general, the reindeer were subjected to a 2-week training program in which they were restrained periodically in individual metabolism stalls (99 x 51 cm) located in an adjacent chamber. These stalls were equipped with stainless steel funnels for the collection of urine; an expanded metal grate above each funnel directed fecal pellets to the side for collection in a 0.3 cm wire mesh reservoir. Feed and water (or snow) were offered on a rack which was built into the front of each stall.

Experimental Protocol

Table I gives the chronological order of temperatures and feeding regimens and the duration of each pre-trial and trial period. The reindeer were allowed the relative freedom of the box stalls for adjustment to each temperature or dietary change. Feed and snow

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 1

Treatment schedule

Trial	Ambient temp. °C	Feed	Dry matter intake, kg/day No. 24	Dry matter intake, kg/day No. 9	Water as:	Pre-trial period, days	Trial period days
A	+10	P	1.90	1.58	W	7	5
B	+10	P	1.07	1.07	W	9	5
C	- 5	P	1.61	1.61	S	11	5
D	- 5	P	1.54	1.61	W	14	5
E	- 5	L	0.82	1.30	S	17	5
F	-20	L	0.81	0.99	S	16	5
G	-20	L	1.04	1.15	S	9	5
H	-20	L	0.99	1.05	W	16	5
I*	-20	L	1.15	1.22	S	17	4
J	-20	P	1.49	1.49	S	25	5
K	-20	P	1.09	1.16	S	6	5
L	+10	P	1.53	1.70	W	30	10

P=Cattle Starter #1, Ralston-Purina Corp., St. Louis, Mo.

L=Hand-picked lichens.

W=Liquid water (5-10°C).

S-Snow (ambient temperature).

*Trace-mineralized salt block (Morton Salt Co., Chicago, Ill.) offered ad libitum.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(where applicable) were stored at the ambient temperature specified for each trial. Liquid water, when offered, was stored at 5-10°C.

At 1130 h on the first day of each trial the reindeer were removed from the box stalls, weighed, and placed in the metabolism stalls. At about 1200 h a 12-ml preinjection blood sample was withdrawn by left jugular venipuncture, into a syringe containing 2-3 drops of ammonium heparin (1000 USP units/ml). Immediately thereafter, 100 μCi of tritiated water (New England Nuclear Corp., Boston, Mass.) in 5 ml of sterile saline were injected into the right jugular vein, also by venipuncture, and the syringe was rinsed three times with venous blood to insure complete administration of the tracer. Half of the daily ration of feed was given at 1230, and water (or snow) was offered for 15-20 minute intervals at approximately 1300, 1500 and 1700 h; any uneaten feed was withdrawn immediately prior to the last watering period of the day. The second half of the 24 h ration was offered at 0830 h on the following day, and water or snow was again provided at about 0900 and 1100 h. At 1200 h the feed was removed, a 12-ml blood sample withdrawn by jugular venipuncture, and the 24 h collection of urine and feces were weighed. A 30ml aliquot of urine and 100-150g of feces were sealed in plastic containers and frozen for later analysis. The amounts of feed and water consumed over each 24-hr period were measured and 6 samples from each lot of feed were withheld for subsequent analysis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Analytical Methods

Recovery and radioassay of plasma water were achieved as described previously in Chapter 1.

Three plasma samples from each trial period were chosen at random for the various analyses (see below), and reported values are means for each group of selected samples. Samples of feed and aliquots of daily fecal collections from each trial were dried at 105°C, to a constant weight (~48 hrs.) for determination of moisture content, bulked on the basis of equal weight, and ground in a Wiley Mill (Authur H. Thomas Co.) through a 0.3 mm mesh screen. Urine samples from each trial were combined in proportion to their 24 h volumes.

Plasma total protein was estimated with a refractometer (American Optical Co.); values were obtained from a scale relating refractive index and concentration of total protein in human serum. Plasma and urinary urea nitrogen were determined using an automated method (Technicon Auto-Analyzer) based on a modified version of the carbamino-diacetyl procedure (Marsh et al. 1965). Osmotic pressure of plasma and urine was determined with a Model 31LA Advanced freezing point osmometer.

Weighed aliquots (~4g) of dry, previously ground feed and feces were ashed in a muffle furnace (550°C for 12 h). The weighed, dry ash was washed into a beaker with deionized water, acidified with ~5 ml of concentrated hydrochloric acid, and heated on a steam bath for 2 h. The hot suspension was filtered into a 100 ml volumetric

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

flask and brought up to volume at room temperature with deionized water. Sodium and potassium concentrations in ash, plasma and urine samples were determined using a Perkin-Elmer Model 303 atomic absorption spectrophotometer.

Gross energy of feed was estimated by burning a 1-2 g sample in a Parr bomb calorimeter. Kjeldahl nitrogen (A.O.A.C. 1960) was determined from a 80-100 mg aliquot of each feed and fecal sample and from 5 ml of each urine sample; protein in feed was estimated as: nitrogen x 6.25. Crude fat was determined as the weight loss of a 4-5 g feed or fecal sample following extraction (6-8 h) with ethyl ether in a Soxhlet apparatus.

All chemical components of feed and feces are given as a percentage of dry matter; carbohydrate was estimated as 100% minus the sum of the percentages of ash, protein and crude fat, and therefore includes lignin as well as cellulose and soluble carbohydrate.

Calculations

The mathematical treatment of the distribution and kinetics of tritiated water in the total body water pool, and the calculation of pool size and turnover have been described previously (Chapter 1).

Daily water and dry matter intake and the corresponding losses in feces were determined from the respective means of wet weight and moisture content. Fecal wet weight was not adjusted for evaporation; the mean moisture content of freshly collected feces was not significantly different from that of 24 h fecal collections where such comparisons were made. Similarly, urine volumes have

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

not been corrected for evaporation; it was assumed that such losses remained at a nearly constant percentage throughout the experiment.

Nitrogen, sodium, and potassium balances were based on their respective mean concentrations in feed, feces and urine; the quantities of sodium and potassium in the liquid water or snow consumed were ignored in the calculations.

Daily production of metabolic water was estimated as the sum of the amounts resulting from the katabolism of protein, fat and carbohydrate, i.e., 0.41, 1.07, and 0.60 ml water per g, respectively (Consolagio et al. 1963). Protein katabolism was calculated as 6.25 times the mean daily rate of urinary nitrogen excretion. The quantities of carbohydrate and fat oxidized were estimated as their apparently digestible intakes.

Statistical Methods

The method of least squares was employed in the characterization of rectilinear and exponential regressions. Significance was evaluated using the Student's t-test; regression equations were compared by analysis of covariance.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

RESULTS

Results of the analysis of feed samples are shown in Table 2 with the specific trial(s) applicable to each. Of particular interest are the significantly higher levels of nitrogen, ash, sodium and potassium (all $p<0.01$) in the pelleted feed compared with those in mixed lichens, while differences in crude fat content and gross energy are not significant.

Measurements of body weight and total body water volume (percentage of body weight) are listed in Table 3A and are shown diagrammatically in Figure 1 where body weight has been partitioned into total water and solids. Body weights decreased steadily during the first three trials and, following a slight increase in trial D, continued decreasing through the first two lichen feeding trials (E and F). Of the approximately 12 kg net decrease in body weight during this interval, reductions in total body water volume account for only about 5 kg, indicating that losses of body solids for both animals were approximately 7 kg. Between trials F and I body weights were maintained and total body water increased, while body solids declined to 16.3 and 11.5 percent of body weight for Nos. 24 and 9, respectively; apparently the lost solids were replaced by an equal weight of water. With the return to a pelleted diet (trial J), body weights and body solids increased sharply, although body water volumes were somewhat higher and body solids substantially lower than those recorded before the commencement of lichen feeding

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 2

Feed Analyses*

Feed	Sample No.	Trial(s)	Moisture, %	Ash, %	Nitrogen, %	Crude fat, %	Sodium, %	Potassium, %	Energy, kcal/g
Pellets	1	A	10.6	9.6	2.09	1.4	0.30	1.7	4.05
	2	B, C	10.6	9.6	2.11	1.5	0.30	1.8	3.94
	3	D	10.6	9.5	2.00	1.8	0.17	1.9	4.14
	4	J. K	17.2	9.4	1.90	1.8	0.18	2.0	4.37
	5	L	10.0	10.2	1.98	2.8	0.18	1.7	4.18
Mean ± S. E.**			11.8 ±1.4	9.7 ±0.1	2.02 ±0.04	1.9 ±0.2	0.23 ±0.03	1.8 ±0.1	4.14 ±0.07
Lichens+	1	E	40.3	2.5	0.50	2.1	0.008	0.067	4.15
	2	F	40.0	2.6	0.62	1.8	0.012	0.067	4.31
	3	G	38.3	2.3	0.39	1.2	0.007	0.065	4.34
	4	H, I	45.2(H), 41.8(I)	2.2	0.39	1.0	0.010	0.066	4.28
Mean ± S. E.			41.1 ±1.2	2.4 ±0.1	0.48 ±0.05	1.5 ±0.3	0.009 ±0.005	0.066 ±0.005	4.27 ±0.04

* Each value represents a mean obtained from the analysis of six subsamples.
**Standard error of the mean.
+ Approximate species composition: Cladonia alpestris, 60%; Cladonia rangiferina, 20%; Cladonia arbuscula 10%; Cetraria islandica, 5%; Cladonia gracilis, Cetraria cucullata, Cetraria laevigata, Stereocaulon alpinum, total of 5%. (courtesy of R. Pegau, Alaska Department of Fish and Game)

NOTE: Except for moisture content, all components are expressed as a percentage of dry weight.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 3A

Changes in body weight, total body water volume, and water flux with variations in dietary regime and ambient temperature

Trial	Reindeer No.	Body weight, kg	Total body water vol., %*	$t_{1/2}$, days	Water flux rate, (ml/day)/kg	Water input, l/day			Water output, l/day	
						preformed	drinking	metabolic	fecal	urinary
A	24	77.5	68.3	2.6	179	0.22	12.99	0.63	2.43	8.34
	9	98.0	68.0	6.3	75	0.19	6.69	0.56	1.33	3.13
B	24	74.5	71.8	3.0	168	0.13	12.22	0.36	1.14	9.33
	9	94.0	72.2	5.9	85	0.13	7.25	0.39	0.87	5.04
C	24	72.5	73.0	5.5	92	0.19	5.80	0.51	1.70	2.99
	9	93.0	76.1	8.3	64	0.19	5.53	0.48	1.45	3.17
D	24	73.0	71.2	5.0	99	0.18	6.71	0.49	1.55	4.28
	9	95.5	71.3	7.4	67	0.19	5.42	0.44	1.70	3.41
E	24	67.5	70.1	25.4	19	0.56	0.77	0.25	0.67	0.38
	9	89.0	69.9	27.9	12	0.88	0.17	0.50	0.72	0.24
F	24	65.0	72.2	34.3	15	0.54	0.28	0.28	0.54	0.38
	9	86.0	72.3	35.4	14	0.66	0.02	0.32	0.74	0.33
G	24	64.5	79.5	32.5	17	0.64	0.19	0.39	0.60	0.22
	9	85.5	77.7	31.8	17	0.71	0.00	0.42	0.68	0.25

TABLE 3A (continued)

Trial	Reindeer No.	Body weight, kg	Total body water vol., %*	$t_{1/2}$, days	Water flux rate, (ml/day)/kg	Water input, l/day			Water output, l/day	
						preformed	drinking	metabolic	fecal	urinary
H	24	64.0	83.3	10.7	54	0.82	2.40	0.33	0.75	2.31
	9	85.0	81.8	31.2	18	0.86	0.28	0.37	0.70	0.64
I	24	65.0	83.7	17.5	33	0.83	1.20	0.40	1.09	0.45
	9	85.0	88.5	27.1	23	0.87	1.26	0.49	0.73	0.84
J	24	72.0	75.8	5.9	89	0.31	5.30	0.40	2.03	3.87
	9	93.0	75.5	7.7	68	0.31	5.31	0.41	2.07	3.64
K	24	69.0	72.8	7.4	68	0.23	3.97	0.32	1.45	3.64
	9	88.0	73.0	10.8	47	0.24	3.17	0.23	1.81	2.19
L	24	71.5	74.0	3.6	143	0.17	8.58		1.47	5.58
	9	83.0	78.1	5.2	104	0.19	6.99		1.47	3.39

*Percentage of body weight.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

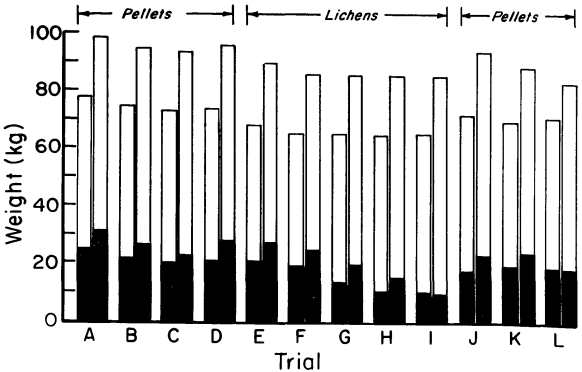


FIGURE 1. Total body water (□), total body solids (■) and body weight (total of water and solids) for reindeer Nos. 24 and 9, respectively.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(trial D). The additional small increases in body solids observed in trial K were accompanied by comparatively large reductions in total body water with the result that body weights declined. In trial L body solids again decreased and total body water volumes were slightly elevated; No. 24 showed a small gain in body weight due to the increase in body water while the rather substantial decrease in body solids by No. 9 resulted in a nearly parallel body weight reduction.

Water flux (ml/day per kg body weight) ranged from 14 at the lowest intake of lichens to 179 at the highest intake of the pelleted ration; the associated $t_{1/2}$ values for tritiated water were 35.4 and 2.6 days, respectively (Table 3A). In considering the influence of dry matter intake on water flux (Fig 2a), two distinctly different regression lines emerge from the results of trials conducted at -20°C with snow offered as the supplemental water source (i.e., trials F, G, J and K). Although these relationships are not statistically significant, a basically different response to feed type is suggested in that, within the range of dry matter intakes, more than three times as much water was turned over per gram of dry matter consumed of the pelleted diet than with that of the lichen diet. Of the major differences found between the two feed types (Table 2), the largest is that of protein content, one of several major factors previously associated with seasonal extremes of water flux in grazing reindeer (Cameron and Luick 1972). Thus, when water flux is considered as a function of nitrogen intake, a single

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

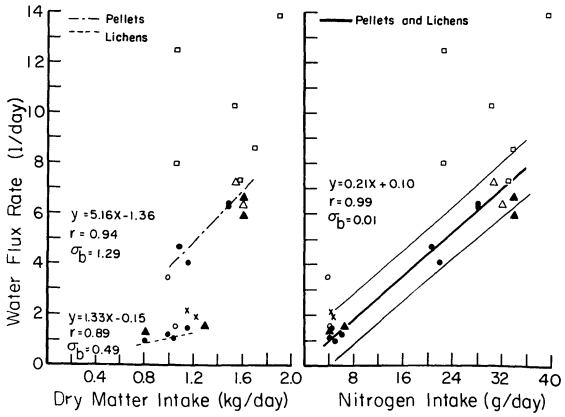


FIGURE 2. Water flux rate as a function of dry matter intake (a) and nitrogen intake (b). The regression lines are based on those trials conducted at -20°C with snow offered as a supplementary water source (i.e., trials F, G. J and K). The 95% confidence limits are shown for the regression line in b.

Legend: +10°C (□); -5°C (△,▲); -20°C (○ ,●); open characters = liquid water, closed characters = snow; X= supplemental salt at -20°C, snow.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

linear regression results from the same observations (Figure 2b) and indicates that the reindeer turned over approximately 230-240 ml of water per gram intake of nitrogen (or 6.25 g protein).

Seven points occur beyond the 95% confidence limits of the relationship between water flux and nitrogen intake. Five points represent trials conducted at +10°C (pellets) and lie above the regression line. These deviations are probably due to augmented rates of insensible water loss at the higher temperature. The response of one reindeer (No. 9) to a temperature of 5°C (pellets; liquid water) was substantially lower than the predicted value; and it is of interest to note that within observations for each animal, generally the provision of liquid water at -5°C (trial D) was associated with an increase in water flux relative to nitrogen intake compared with the corresponding result obtained when only snow was supplied (trial C). Similarily, liquid water supplementation at -20°C (trial H) stimulated disproportionate increases in water flux such that one point occurred well above the confidence limits of the regression line. It is also noteworthy that the consumption of supplemental minerals (trial I) was associated with elevated water flux rates, although the increases were not significant.

Analytical results of feces and urine samples are shown in Table 3B. The mean moisture content of fecal samples from trials in which pellets were consumed (70.4%) was significantly different ($p<0.01$) from that associated with lichen-feeding trials (60.6%). The

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 3B

Analyses of feces and uri
and apparent digestibilit

Feces

Trial	Reindeer No.	Moisture, %	Ash, %	Nitrogen, %	Crude fat, %	Sodium, %	Potassium, %	Nitro g/l
A	24	76.5	15.2	1.56	1.5	0.067	0.35	3.8
	9	70.8	15.8	1.44	1.6	0.099	0.37	8.6
B	24	71.9	15.4	1.46	1.4	0.023	0.16	2.3
	9	69.3	16.3	1.45	1.2	0.006	0.16	4.6
C	24	71.5	15.6	1.43	1.5	0.017	0.25	8.3
	9	66.0	16.0	1.44	1.3	0.013	0.11	7.5
D	24	70.5	15.6	1.54	1.5	0.019	0.12	4.6
	9	67.8	16.5	1.34	0.9	0.051	0.11	4.3
E	24	61.1	6.2	1.34	0.8	0.029	0.50	3.4
	9	58.7	7.2	1.54	0.6	0.018	0.33	5.9
F	24	59.1	7.0	1.56	0.8	0.046	0.79	5.5
	9	60.2	6.8	1.59	0.6	0.087	0.35	6.6
G	24	59.1	5.9	1.51	0.7	0.076	0.26	4.4
	9	58.4	6.3	1.42	1.0	0.101	0.24	6.3

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 3B

ıalyses of feces and urine,
ıd apparent digestibilities

		Urine				Apparent Digestibility, %	
Potassium, %	Nitrogen, g/l	Urea-N, mg/l	Sodium, g/l	Potassium, g/l	Osmotic pressure, mOs/l	Dry matter	Organic matter
0.35	3.86	2.46	0.503	3.36	360	60.5	63.0
0.37	8.60	6.38	1.769	6.33	650	65.2	67.6
0.16	2.37	1.73	0.141	1.75	180	58.9	61.5
0.16	4.60	3.34	0.633	3.22	320	64.5	67.1
0.25	8.31	6.38	0.925	8.02	880	57.8	60.5
0.11	7.58	5.95	0.482	7.30	740	54.0	57.3
0.12	4.62	3.21	0.141	5.05	360	57.8	60.6
0.11	4.30	6.58	0.241	7.30	610	50.3	54.2
0.50	3.44	2.34	0.015	0.20	260	47.6	49.6
0.33	5.97	1.58	0.017	0.97	430	61.5	63.4
0.79	5.58	1.15	0.010	0.34	360	53.1	55.3
0.35	6.64	0.15	0.016	1.55	480	50.5	52.6
0.26	4.44	0.27	0.010	0.08	370	60.6	62.0
0.24	6.36	0.16	0.023	0.34	480	57.4	59.2

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 3B (continued)

Feces

Trial	Reindeer No.	Moisture, %	Ash, %	Nitrogen, %	Crude fat, %	Sodium, %	Potassium, %	Nitrog g/l
H	24	61.2	6.4	1.36	0.9	0.079	0.26	0.5
	9	59.6	7.1	1.60	1.1	0.120	0.27	2.3
I	24	66.8	6.1	1.47	0.7	0.308	0.24	7.3
	9	61.5	7.2	1.45	1.0	0.286	0.16	5.7
J	24	70.1	14.0	1.44	1.3	0.020	0.18	8.2
	9	71.9	14.7	1.32	1.4	0.021	0.22	7.9
K	24	70.7	16.9	1.44	1.3	0.030	0.21	7.5
	9	68.2	15.4	1.52	0.9	0.016	0.20	10.1
L	24	71.4	17.9	1.26	1.3	0.019	0.13	---
	9	69.6	17.5	1.42	1.5	0.009	0.12	---

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE 3B (continued)

Potassium, %	Nitrogen, g/l	Urea-N, mg/l	Urine Sodium, g/l	Urine Potassium, g/l	Osmotic pressure, mOs/l	Apparent Digestibility, % Dry matter	Apparent Digestibility, % Organic matter
0.26	0.55	0.13	0.005	0.02	60	52.5	54.5
0.27	2.34	0.54	0.014	0.28	190	54.3	56.6
0.24	7.36	1.33	3.950	0.44	840	53.0	54.9
0.16	5.73	1.95	4.774	0.28	980	63.1	65.0
0.18	8.22	5.73	1.266	7.06	680	42.3	45.2
0.22	7.91	6.98	1.246	6.81	730	45.6	48.8
0.21	7.59	6.70	0.985	5.59	610	45.0	49.5
0.20	10.14	9.18	0.915	8.02	800	27.6	32.4
0.13	---	---	---	---	---	61.4	64.8
0.12	---	---	---	---	---	62.4	65.4

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

respective means of fecal ash were 15.9 and 6.6% which also differed significantly ($p<0.01$). Fecal nitrogen concentration was constant within and between dietaries with an overall mean of 1.45% (coefficient of variation, 6.2%), while urinary levels were quite variable and demonstrated no apparent uniformity in response to the various treatments. No trends were noted between sodium and potassium concentrations in feces and feed type or temperature, but the urinary levels, in general, reflected the respective concentrations in the associated feed types. Urinary and fecal concentrations of sodium were appreciably higher during mineral supplementation (trial I). In general, urinary urea nitrogen was elevated during the higher intakes of nitrogen which accompanied the feeding of pellets. Urine osmotic pressure varied from 60 to 980 mOs/l and, although the mean was highest during trial I, the values appeared unrelated to urine volumes.

The apparent digestibilities of dry matter and organic matter (Table 3B) declined during the initial pellet feeding trials (A-D) and, while individual values varied over a range of approximately 15%, the means remained slightly lower through the period of lichen feeding (trials E-I). With a return to the pelleted diet (trial J), apparent digestibilities decreased substantially, but in trial L were restored to levels similar to those obtained at the commencement of the experimental series. Presumably, these latter changes reflect an initially reduced activity of the rumen microfloral population and its subsequent adaptation to the dietary change from lichens to pellets, although it is noteworthy that similar variations

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in digestibility did not accompany the introduction of lichens (trial E) following a period of feeding pellets.

Figure 3 shows the variations in some plasma constituents and the balances of nitrogen, sodium and potassium over the experimental period. Of the plasma parameters, the most striking change was that in urea nitrogen concentration; mean values associated with the pelleted and lichen diets were 5.1 and 35.5 mg/100 ml of plasma, respectively, with extremes ranging from 2.3 to 39.2 g/100 ml of plasma in one reindeer (No. 9). Plasma total protein (g/100 ml of plasma) declined steadily from an initial mean of 7.0 to a low of 5.9 in the final lichen feeding trial, and subsequently increased to a final mean of 6.4. Plasma sodium and potassium concentrations were approximately constant during the first four trials, but with the commencement of lichen feeding sodium levels increased by about 10 meq/l while potassium concentrations declined slightly, with the result that the mean sodium:potassium ratio reached a peak in trial F at nearly twice the initial value. Both sodium and potassium concentrations increased during the lichen feeding experiments to means of approximately 170 and 5.5 meq/l, respectively, and thereafter decreased to, or remained at, values similar to those recorded for trial A.

With the exception of trial D, negative nitrogen balances were obtained in all treatments (not determined for trial L) and became progressively more negative toward the end of the experimental series (Figure 3). Values for trials J and K indicate a daily loss of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

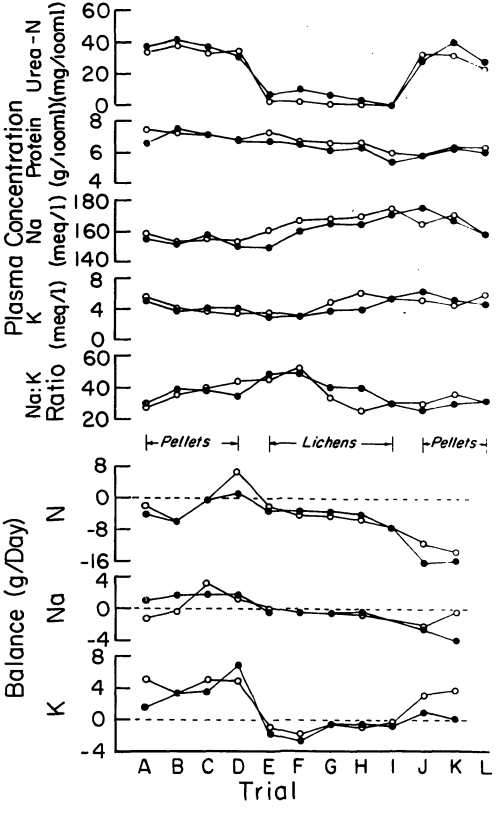


FIGURE 3. Changes in the concentration of some constituents in plasma and the balances of nitrogen, sodium and potassium for reindeer Nos. 24 (●) and 9 (o).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

nearly 16 g of nitrogen and correspond to extremely low apparent digestibilities of organic matter (Table 3B). These deficiencies were accompanied by a paradoxical increase in body solids (Figure 1). Sodium balances were much less variable than those of nitrogen but followed a similar trend. It is of interest to note that net sodium losses were maintained near zero at sodium intakes as low as 70 mg/day (lichens), whereas daily intakes of nearly 3 g (pellets) in several cases resulted in substantially greater net losses of sodium. The values for potassium balance reflected the extremes of potassium content of the two feed types and were negative only in those trials in which lichens were consumed. Although appreciable losses of nitrogen and sodium are shown for the last two trials, a net gain of potassium is indicated despite the relatively low apparent digestibilities of both dry matter and organic matter (Table 3B).

The regression relating water flux determined from TOH dilution, and the sum of measured water consumption and calculated metabolic water production is shown in Figure 4. The slope of unity with a small standard deviation, together with the high correlation coefficient and a Y-intercept which is not significantly different from zero, indicate that the technique is highly reliable for estimating water kinetics, and further that its accuracy is unaffected by extremes of diet, temperature or body condition.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

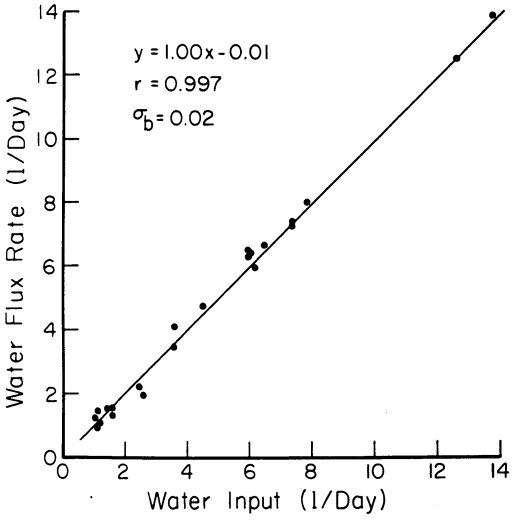


FIGURE 4. The relationship between water flux determined by TOH dilution and the total water input, i.e., the sum of measured water intake plus calculated metabolic water production (trials A-K).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DISCUSSION

Changes in Body Composition

As mentioned in the previous chapter, the prediction equations for body composition proposed by both Pace and Rathbun (1945) and Panaretto (1963) provide unrealistic percentages of body fat when applied to reindeer. From the relationship between in vivo total body water determinations and carcass analysis, Searle (1970) has established equations for the estimation of fat and protein content in sheep which appear to supply more credible values. Where justified, these equations have been employed in the following discussion.

The reduced intake of pellets in trial B relative to that in trial A (Table 1), was associated with a decline in body solids (Figure 1), primarily as fat (4-5 kg) but to a smaller extent as protein (70-100 g), as indicated by the use of Searle's equations. Since the percentages of fat and water tend to be inversely related in the animal body (Pace and Rathbun 1945), the observed increases in percentage total body water (Table 3A) support the occurrence of body fat utilization; and body protein losses are further indicated by the relatively greater daily losses of nitrogen in trial B (Figure 3). In addition, it is of interest that slightly reduced apparent digestibilities of dry matter and organic matter (Table 3B) accompanied the decreased feed intakes at +10°C.

Further decreases in body weight and body solids occurred with the temperature change to -5°C in trial C (Figure 1), and increments

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in total body water relative to body weight indicate continued losses of body fat. Searle's equations show this decrease as being on the order of 1 and 4 kg for Nos. 24 and 9, respectively; but further indicate that although No. 24 continued to decline in body protein, No. 9 increased in body protein content by nearly 300 g. This latter value, in light of the slightly negative balance of nitrogen (Figure 3), is probably incorrect and is apparently due to the body water increase of about 3 l (Figure 1). Normally, the amounts of water and nitrogen in the animal body are directly related (Panaretto 1963), and the equations of Searle reflect this relationship. It seems more likely that a disproportionate accumulation of water occurred in No. 9, perhaps as a result of increased sodium and potassium retention (Figure 3), and that body protein was in fact decreasing during this period. Despite small to moderate decreases in the digestiblity coefficients, the reduced ambient temperature of trial C is associated with a relative improvement of nitrogen status (Figure 3).

The provision of liquid water at the same ambient temperature (trial D) was associated with increases in body solids of approximately 1 and 5 kg for Nos. 24 and 9, respectively; both animals were in positive nitrogen balance (Figure 3). Searle's equations however, predict a slight decrease in the body protein content of No. 24, but the calculated values for No. 9 are in general agreement with the nitrogen balance value which was higher than any previously or subsequently recorded. In addition, both reindeer were in positive

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sodium and potassium balance. Values for body fat increased relative to estimates for either trial B or C and corresponded to the lower percentages of total body water (Table 3A). The apparent digestibilities of dry matter and organic matter however, did not reflect the improvement in body condition and either remained constant or declined slightly (Table 3B); despite the improvements in nitrogen status, the apparently digestible intake of nitrogen was slightly lower than in the preceding trial. Thus, it appears that the beneficial effects of consuming liquid water were not due to increased rates of ruminal fermentation or microbial protein synthesis, but apparently resulted from a more efficient utilization of metabolic intermediates and (or) a nitrogen conserving mechanism operating subsequent to nutrient absorption from the alimentary tract.

With the commencement of lichen feeding at -5°C (trial E) decreases in body solids were very small but total body water was reduced by 5-6 l (Figure 1). The calculated amounts of body protein were approximately 1 kg lower for both reindeer which seems excessive considering the relatively small daily losses of nitrogen. Estimates of fat content showed a slight increase which is reflected by the decrease in total body water as a percentage of body weight (Table 3A). Thus, it appears that energy demands were satisfied or slightly exceeded by the diet while the protein content of lichens was insufficient for maintenance of nitrogen equilibrium. Although the two reindeer responded similarily with regard to changes in body composition, it is of interest that, compared with trial D, No. 9 showed a 10-unit increase

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

in the apparent digestibility of both dry matter and organic matter while the same parameters for No. 24 decreased by about the same amount.

In trial F ambient temperature was lowered to -20°C. The absolute quantities of body water remained essentially constant in body animals so that the reduced body weights were entirely due to losses of body solids (Figure 1). The increases in total body water of from 70 to 72% of body weight (Table 3A) indicate body fat reductions which, according to Searle's equations, amounted to 2 and 3 kg for Nos. 24 and 9, respectively; body protein losses were on the order of 200 g for both animals. Thus, it appears that digestible dry matter intake was inadequate to meet the increased energy demands encountered with the decreased ambient temperature, with the result that large amounts of body fat were mobilized while body protein katabolism proceeded at a rate which, as in the previous trials, resulted in a daily loss of 3-4 g of nitrogen.

Trials F through I were characterized by the maintenance of body weight with a progressive loss of body solids. It is in these experiments that the use of Searle's equations become of only limited value; for although the increasingly greater percentages of total body water (Table 3A) qualitatively affirm the calculated reductions of body fat, the absolute increases in body water content (Figure 1) result in estimated net increases in protein. Considering the consistently negative balances of nitrogen obtained during this interval and the decreasing concentrations of plasma total protein

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(Figure 3), the latter result is absurd. Also, because of the above overestimates of protein content, the calculated fat values are actually in excess of total solids losses. It can be calculated from the daily losses of nitrogen for individual trials and the number of days applicable to each value, that the total loss of body protein between trials F and I was approximately 1.6 and 1.8 kg and that, by difference, body fat decreased by an estimated 5.9 and 12.2 kg for Nos. 24 and 9, respectively. The greater loss of body substance by No. 9 is possibly a result of a slightly lower feed intake in relation to body size. The digestible dry matter intake for No. 9 during the period in question was approximately 22 g/day/$kg^{3/4}$, while that of No. 24 averaged 24 g/day/$kg^{3/4}$.

The process of progressive water accumulation described above remains as an unusual and interesting phenomenon. Results of recent carcass analyses of three yearling reindeer (Luick, unpublished) may assist in defining the change. Two of the yearlings were fed hand-picked lichens for 8 weeks and the third served as a control and was allowed an ad libitum intake of the same pelleted ration as that used in the present study. The body weights of all three reindeer declined progressively during the first three weeks and, as in trials F through I, subsequently stabilized through the remainder of the experiment, although net weight losses were much greater in the lichen-fed animals than in the control. Based on the means of plateau body weight, dry matter intake of lichens was regulated at amounts equal to 32 and 46 g/day/$kg^{3/4}$ (comparable

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

intakes in the present study were 40-44 g/day/kg$^{3/4}$). Measurements made at the time of slaughter indicate that, while total alimentary water in the control animal amounted to 15% of body weight, the same value for the two reindeer on the lichen diet was 26%. Assuming that it is justified to relate the value for alimentary water content of the control to the results of trial F and those of the lichen-fed yearlings to the observation in trial I, it can be calculated that for Nos. 24 and 9, respectively, approximately 100% and 70% of the increases in body water can be accounted for by an expansion of the alimentary water component. Similarly, Coady and Gasaway (1972) reported a higher percentage of rumen water in winter-killed moose (12.9% of body weight) compared with that in moose collected in late Spring (7.6% body weight), although dry matter percentages of rumen contents were higher in the former (17.6%) than in the latter (13.0%). Such changes in rumen fill are probably related to forage quality. Relatively indigestible feeds, containing a high proportion of fiber, require a longer retention time in the rumen in order to compensate for the slower rate of fermentation (Hungate 1966), and the maintenance of a greater amount of substrate in the rumen might tend to minimize decreases in the total production rate of volatile fatty acids and the synthesis of microbial protein.

It is obvious that increases in total body water not due to changes in the amount of gut water must result from tissue hydration, and there is some evidence for such an occurrence in other species. For example, Siebert and Macfarlane (1969) found that Shorthorn

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cattle in the tropics increased in body weight by 36 kg between winter and summer, but gained 50 kg of body water, indicating a 14 kg loss of body solids. These workers suggested that the body water increase was due the combined effects of summer heat and poor supplies of food, and probably arose from an expansion of the gut and extracellular water spaces. Morris et al. (1962) reported that sheep shorn in winter and kept at high stocking rates lost 3.7 kg in body weight but gained 2.1 l in total body water, with the result that body solids decreased by 5.8 kg. The volumes of extracellular fluid and plasma increased by 710 and 260 ml, respectively, and the total of gut and intracellular water, estimated by difference, was enlarged by 1.4 l; cold exposure and undernutrition apparently combined to produce these changes.

Sheep grazing very wet pastures lose body weight but show significantly higher percentages of total body water, extracellular fluid and plasma volumes (Macfarlane et al. 1966). Macfarlane and co-workers (1966) concluded that although the increases in body water could have been due in part to greater relative volumes of alimentary water, on the basis of body weight 61-65% of the total water increase was due to a higher volume of extracellular fluid; and further, since only a slight increment in plasma volume was noted, approximately one-half of the total increase was accounted for in the interstitial fluid spaces. These observations are associated with a low plane of nutrition induced by a submaintenance intake of dry matter while water was probably supplied in excess of the requirement. Cameron and Luick (1972) reported similar changes in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reindeer which were ostensibly grazing forage of high water content (see previous chapter). The fat and cellular tissues which disappear during undernutrition are apparently replaced by water which is held in the extracellular fluid spaces (Macfarlane et al. 1959) although the mechanism remains undefined.

Results of the present study show that between trials F and I the concentration of plasma sodium, and to a smaller extent that of plasma potassium, rose with the increasing volumes of body water (Figures 1 and 3). Since an increase in the total amount of circulating sodium or potassium is improbable considering the consistently negative balances of these cations (Figure 3), the higher concentrations may indicate a shrinkage of plasma volume. Further, if it is assumed that the concentrations of sodium and potassium in the plasma reflect those in the entire extracellular fluid, a reduction of the interstitial fluid volume is also indicated. Hence, it appears unlikely that the net increases in total body water are due to an accumulation of water in the extracellular fluid, but the possibility of an increased intracellular water pool remains open to speculation. For example, the replacement of mobilized fat by water in adipocytes proposed by Farrell (1970) for undernourished sheep is a possibility, but such a phenomenon could account for only a small portion of the total increase in water content observed here.

Thus, the evidence indicates that the alimentary pool is quantitatively the most likely site of water accumulation, and that the observed effect may result from long term exposure to dietary

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

insufficiency. A larger volume of alimentary water in general, and of rumen water in particular, has probable adaptive significance in a cold environment, and would operate as a thermal buffer which would tend to minimize fluctuations in body temperature induced by the consumption of snow and frozen forage. In a similar manner, ruminants indigenous to a hot climate are able to retain large quantities of rumen water which is drawn upon in resisting the deterimental effects of water deprivation and (or) excessive losses of water by evaporation (Macfarlane 1964). Indeed, the rumen has been referred to as a "water store" in sheep (Hecker et al. 1964).

The shift from lichens to a comparatively high dry matter intake of the pelleted ration in trial J was associated with an initial increase in body weight which subsequently declined in trial K. Total body solids showed initial increases of 6 and 13 kg for Nos. 24 and 9, respectively; but unlike the body weights, continued to rise slightly in trial K (Figure 1). Values obtained using Searle's equations and the reductions in percentage body water (Table 3A) together indicate substantial increases in fat content. By trial K it is estimated that body fat was nearly restored to levels obtained for the reindeer in trial F. The calculated amounts of body protein show an increase relative to those for trial F, but such an estimate is unreliable considering the consistently negative balances of nitrogen (Figure 3). Therefore, it appears that the dietary change from lichens to pellets at -20°C precipitated a reduction in body water and a further decline in body protein while body fat accumulated.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

It is interesting to note that, although the balances of potassium increased to positive values in response to higher potassium intakes, the net losses of sodium (Figure 3). The lower digestibilities of dry matter and organic matter (Table 3B) may reflect the inability of the rumen microbial population, previously adapted to a lichen substrate, to ferment the pelleted diet. However, it is equally possible that the low ambient temperature per se was the factor of major influence, since a return to +10°C in trial L resulted in digestibility coefficients similar to those obtained in trials A and B. Similarly, a temperature decrease from +10 to -5°C (trials A and B vs. C and D) resulted in lower digestibilities of the pelleted ration, although the changes were much smaller. In contrast to these results, Johnson and Yeck (1964) reported that the consumption of total digestible nutrients by cattle decreases with increasing ambient temperature between 2 and 35°C. Hence, the effect is confounded by several variables, and any interpretation at this point is equivocal.

Despite the increases in apparent digestibility during trial L, the higher temperature was associated with a loss of nearly 4 kg of body solids by reindeer No. 9. The total solids of No. 24 remained essentially unchanged, and values obtained using Searle's equations indicate that losses of body fat were approximately balanced by an increase in body protein. In contrast, estimates for No. 9 show that, in addition to a more than 5 kg loss of body fat, approximately 200 g of body protein was katabolized. Although

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the increased percentages of total body water (Table 3A) support the calculated reduction of body fat, nitrogen balance data are not available for verification of the estimated change in protein content. It is apparent however, that the increased temperatures of trial L were associated with an appreciable reduction of body tissues, and further that this effect was detectable even after a 30-day exposure to the higher temperature (Table 1).

The parameters of body composition obtained for trial L must be regarded as static in nature and, as such, cannot be truly indicative of what is obviously a dynamic process. The data do not exclude the possibility that during the pre-trial acclimation to +10°C, body solids were reduced even further than indicated, and that the results of trial L are merely observations which reflect the process of body improvement subsequent to successful temperature adjustment.

Variations in Water Flux

The direct relationship between water flux rate and the intake of dry matter for each of the two feed types (Figure 2A) is similar to that reported for other ruminants. In sheep, linear correlations have been established between water consumption and the intakes of both dry matter (Calder et al. 1964; Forbes 1968; McIntyre 1970) and digestible organic matter (Morris et al. 1962). Forbes (1968) showed significant differences in total water intake per unit of dry matter intake between sheep fed cubed grass, hay, and silage, and suggested that different contents of nitrogen or soluble ash of the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

rations may account for the dissimilar responses. McIntyre (1970) eliminated nitrogen as having a major influence, for at the same rate of dry matter intake, water intake rose only slightly with increasing nitrogen content of the diet; and since rates of water consumption rose more sharply relative to nitrogen intake when increasing amounts of a high quality feed were given, it was concluded that water intake more closely reflects the intake of dry matter than that of nitrogen.

The water intake of steers has been shown to be substantially higher on high quality diets than at the same dry matter intakes of low quality diets (Vercoe 1967). Other results with cattle by Siebert (1971) denote a linear correlation between the daily turnover of water and organic matter consumption and, in addition, indicate that when the proportion of legumes in the ration was increased at a constant daily feed intake, higher fluxes of water resulted. It was concluded by Siebert that, although water intake is definitely influenced by feed quality, the major effect is that of organic matter intake.

None of the above results for sheep or cattle show unequivocally that any one of the suspected factors (i.e., dry matter, organic matter, nitrogen, or ash) is soley responsible for variation in water flux. In the present experiments the correlation between water flux and nitrogen intake (Figure 2b) suggests causation, but does not exclude the influence of dry matter intake since for a given diet the intakes of dry matter and nitrogen are directly related. If the regression

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

lines shown in Figure 2a are extrapolated to the full range of dry matter intakes, the effect of an increasing nitrogen intake on water flux can be evaluated at equal intakes of dry matter. Thus, for dry matter intakes between 0.8 and 1.6 kg/day, the increase in water flux per unit increase in nitrogen intake ranges from 150 to 200 ml/g nitrogen (or 71 to 95% of the slope in Figure 2b). This ratio approached an asymptote of approximately 210 ml/g nitrogen, which is identical to the slope of the regression line shown in Figure 2b. Although it is apparent that nitrogen intake is not the exclusive determinant of the water flux rates observed at -20°C, these considerations provide evidence that it is a factor of major influence. Hence, the relationship is used as a basis for comparison with results obtained at the higher temperatures.

The disproportionately high water flux rates observed at +10°C in the present study are similar to results reported elsewhere. Studies with cattle by Winchester and Morris (1956) indicate that between -12.2 and +4.4°C water consumption per unit of dry matter intake remains fairly constant, but increases at an accelerating rate thereafter. In fact, Razdun et al. (1971) suggested that the intakes of dry matter and protein may not be important determinants of water intake by Zebu cattle and buffaloes, but that the effects of a hot climate may have relatively more influence. To evaluate the changes in water flux relative to feed intake at the various temperatures, the individual avenues of water loss and the factors influencing each will be examined.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A. Fecal water loss.

If the daily outputs of fecal water are plotted as a function of fecal nitrogen excretion, a linear relationship is seen within data obtained at -20, -5, and +10°C (Figure 5). Analysis of covariance indicated a homogeneity in the regression coefficients of all three lines, but the adjusted means of the lines at +10 and -20°C were significantly different ($p<0.05$). The adjusted mean for the relationship at -5°C was not significantly a different from either of those at +10°C or -20°C. Thus, at a given level of fecal nitrogen excretion, the additional 400 ml of water eliminated via the feces at +10°C, compared with the amount voided at -20°C, is statistically significant. Unfortunately, it is not possible to determine unequivocally if this increase is due to ambient temperature per se or whether it results from the consumption of liquid water, but the points representing trials in which liquid water was offered at -20°C do not deviate appreciably from the regression line for that temperature, which suggests that environmental temperature, and not the form in which water was provided, exerted the major influence.

In considering the various components of fecal dry matter (Table 3B), the relative constancy of nitrogen concentrations throughout the various treatments is particularly striking, and implies a regulation of fecal nitrogen losses. A uniform concentration of fecal nitrogen has been reported in both sheep and deer (Maloiy et al. 1970), and such levels were apparently independent of nitrogen

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

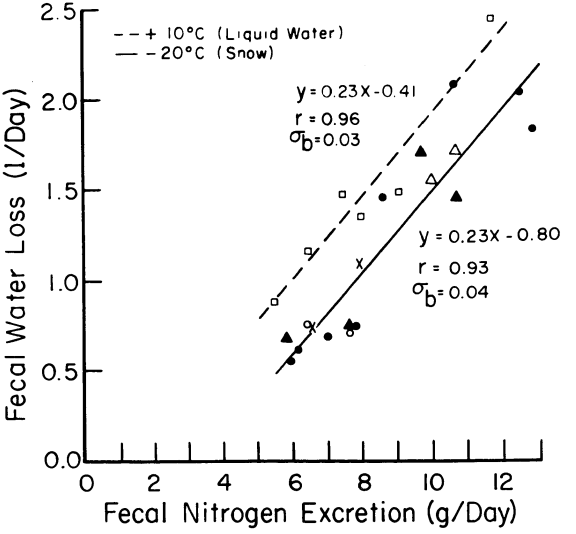


FIGURE 5. Relationships between fecal water loss and fecal nitrogen excretion. The legend is given in Figure 2.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

intake and water consumption. It appears that while fecal nitrogen concentration is regulated independent of nutritional treatment or temperature exposure, the ratio of water to both nitrogen and dry matter in the feces is temperature dependent. Thus, the higher rates of fecal water loss observed at +10°C were due to an increased water content of feces rather than to higher rates of fecal output. This observation is in agreement with data for cattle; Johnson and Yeck (1964) noted that fecal water content increased progressively between 2 and 35°C.

In the present study the mean water content of feces (Table 3B) from trials conducted at +10°C (71.6%) was significantly ($p<0.01$) higher than that associated with trials at -20°C (63.9%). However, it is not completely clear that the increase in fecal water content at +10°C was exclusively an effect of temperature, since if the comparison is restricted to experiments involving the pelleted ration, the mean fecal water content at +10°C is only slightly higher than the value at -20°C (70.2%) and the difference is no longer significant. Possibly the upper temperature to which the reindeer were exposed was not adequately high to elicit the response observed in cattle; unfortunately, the effect is not readily interpretable due to the confounding influence of feed type.

Augmented losses of fecal water relative to those of dry matter and nitrogen represent an important means of eliminating additional water from the body in response to a need for heat dissipation. Furthermore, such a mechanism would not necessitate an increased

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

removal of dry matter, and would thereby tend to maintain the level of nutrient digestibility.

The linear relationship shown in Figure 5 is similar to that noted in cattle. Steers held at temperatures of from 18 to 27°C and fed two levels of nitrogen at both ad libitum and restricted intakes of water demonstrated a direct relation between the amounts of water and nitrogen excreted in the feces (Utley et al. 1970b). In this latter study the slope of the regression line relating fecal water and fecal nitrogen output was identical to that in Figure 5, but the y-intercept was substantially lower. This indicates that at even higher ambient temperatures than those in the present study, fecal losses of water relative to those of nitrogen are lower in cattle than in reindeer. This can be also interpreted as showing that reindeer void less nitrogen by fecal means than cattle at equal rates of fecal water loss. Reduced losses of fecal nitrogen may be important in nitrogen conservation and of particular value in the successful use of low quality feeds (Sperber 1968).

B. Urine volume.

Urinary water loss was found to be highly correlated with total water flux in the present study (Figure 6). The relationship was not affected by temperature, feed type, dry matter intake, or the form in which supplemental water was provided. A similar relationship has been obtained for sheep which were fed a constant ration and held at a temperature of 22°C (Anand and Parker 1966); the slope and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

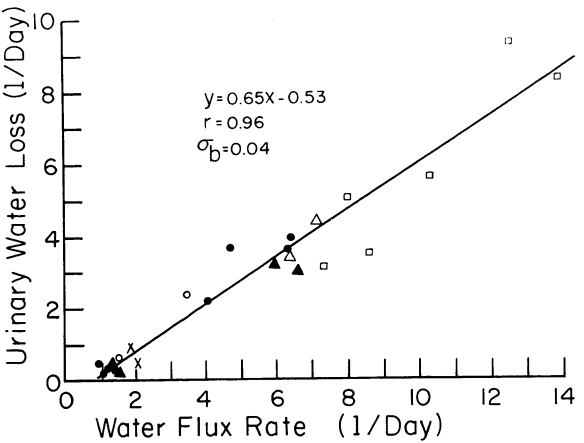


FIGURE 6. The relationship between daily urine volume and water flux. The legend is given in Figure 2.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

y-intercept of the regression calculated for sheep approximate those of the relationship shown in Figure 6, indicating that reindeer, under the conditions of the present study, responded similarly to sheep which were exposed to a higher temperature. Steers housed at temperatures between 18 and 27°C and given various quantities of water and dietary nitrogen likewise demonstrate a linear relationship between urine volume and water consumption (Utley et al. 1970b). Again the slope of the regression line is similar to that obtained for reindeer, but the y-intercept is substantially lower. Therefore, the volume of urine produced by steers is somewhat less in relation to total water flux than that of either reindeer or sheep, and is relatively less important as an avenue of water loss.

Utley et al. (1970a) suggested that higher urine volumes accompanying increased nitrogen intakes in steers result from a need for renal elimination of additonal nitrogen, yet as pointed out above, their data show that urine volume increases linearly with water consumption whether or not water is restricted. The implication is that within certain undefined limits, the quantity of water eliminated by the kidney is pre-determined by the amount of water consumed. In the present study, urine osmolality was highest when supplemental minerals were offered (trial I) and corresponds to high urinary concentrations of sodium (Table 3B), yet the representative points do not deviate appreciably from the regression line in Figure 6. In contrast, the provision of liquid water at -20°C (trial H) was associated with a substantial increase in water flux for reindeer No. 24 relative to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

that predicted by nitrogen intake (Figure 2b), but again the urine volume was consistent with the established regression (Figure 6) despite the extremely low value for urine osmolality (Table 3B). Thus, the correlation shown in Figure 6 is apparently of physiological significance and when interpreted in conjunction with other results, suggests that urine volume is stoichimetrically fixed to water intake and may therefore not be under active control in reindeer, or perhaps even in ruminants in general.

C. Insensible losses of water.

As suggested previously in this report, augmented rates of insensible water loss may account for the higher water flux rates observed at +10°C (Figure 2). In the arid tropics, cattle increase their rates of water turnover during summer heat as compared with winter, and this change is thought to be mainly a function of the additional water required for evaporative cooling (Siebert and Macfarlane 1969). Harbin et al. (1958) obtained a highly significant linear relationship between water consumption per unit body weight and temperature (10 to 35°C) in cattle, while humidity had no significant effect at a constant temperature. Similarly, Forbes (1968) reported a linear correlation between total water intake per unit of dry matter intake and environmental temperature (1-8°C) in sheep.

In theory, the difference between the daily water flux and the sum of the 24 h outputs of fecal and urinary water represents

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the total loss due to evaporation. Since sweating has not been observed in reindeer up to temperatures of 43-45°C (Rosenmann and Morrison 1967), it is likely that respiratory evaporation is the primary avenue of insensible water loss in this species, as it is in sheep (Macfarlane 1964). Because evaporative losses were not measured directly in the present study, calculated values are referred to as "indices of insensible water loss". The pooled means at each temperature differ significantly ($p<0.01$) and increase in a curvilinear fashion with increasing ambient temperature (Figure 7), reaching maximum value of 2.8 l/day at +10°C. Some confidence can be placed in this relationship, for Johnson and Yeck (1964) conducted water balance studies with three breeds of cattle and determined vaporization directly with increasing temperature between 2 and 35°C. If their values are plotted similarly the shape of the resulting curve resembles the relationship in Figure 7.

In summary, the results indicate that at ambient temperatures of -5 and -20°C, water flux in reindeer is primarily determined by the intake of nitrogen. Nitrogen intake apparently manifests its influence through the feces by virtue of the direct relationship between the excretion rates of nitrogen and water. Insensible losses of water are directly related to ambient temperature and increase approximately four-fold between -20 and -5°C, while urinary excretion of water is a function of water flux (intake) and is temperature-independent. At +10°C the augmented rates of water flux chiefly

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

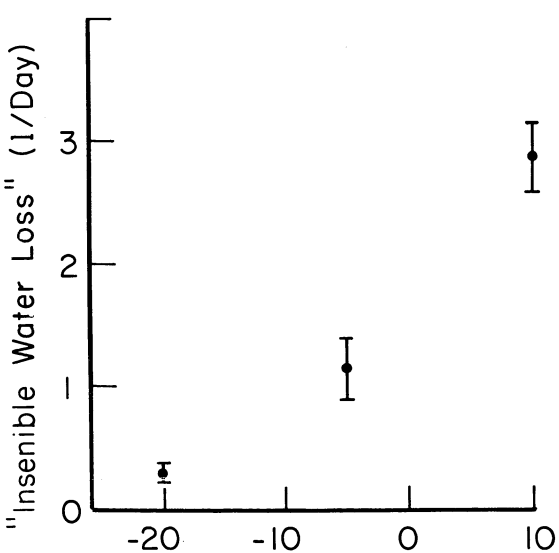


FIGURE 7. Mean (±S.E.) insensible water loss plotted as a function of ambient temperature. Insensible water loss was estimated as the difference between water flux and the total of fecal and urinary water losses.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reflect the increased evaporative losses of water and to a lesser extent an increase in fecal excretion of water relative to that of nitrogen.

Nitrogen and Energy Relations

The dietary shift from lichens (trial I) to pellets (trials J and K) was associated with a decline in the apparent digestibility of both organic matter (Table 3B) and nitrogen. Since the quantity of energy made available during rumen fermentation is likely to be related to the rate of synthesis of microbial protein (Weston and Hogan 1968), these observations probably reflect a reduced capacity of the rumen microflora to digest energy substrates following the change in feed type, with the result that smaller amounts of dietary nitrogen were incorporated into microbial protein, and relatively larger quantities were excreted in the feces. While the mean nitrogen retention for trials J and K was the lowest recorded, that for the lichen feeding trials equalled or slightly exceeded the values obtained in trials A and B (Figure 3). Without exception, nitrogen intakes were lowest when lichens were consumed, and it is tempting to suggest that the lichen diet provided an adequate, or even excessive amount of energy, resulting in a relatively more efficient utilization of dietary nitrogen. The mechanism may involve the optimization of bacterial protein synthesis with minimal losses of ammonia from the rumen (Weston and Hogan 1968). Provided sufficient energy is available, such losses of ammonia are smaller when dietary sources of nitrogen

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

are in short supply than when the nitrogen content of the feed is higher (Sperber 1968); the low concentrations of urea nitrogen in plasma during lichen feeding (Figure 3) may reflect the minimization of such losses and a consequent conservation of ruminal nitrogen.

It has been shown by several workers that plasma urea concentration in ruminants tends to be directly related to nitrogen intake (Maloiy et al. 1970; McIntyre 1970; Nolan et al. 1970). In the present study urea nitrogen concentrations in plasma were significantly ($p<0.01$) higher when the pelleted ration was consumed compared with those associated with lichen feeding. In addition, nitrogen intake by sheep has been shown to be related to the urinary excretion of urea nitrogen (McIntyre 1970; McIntyre and Williams 1970) which in turn is directly correlated with plasma urea nitrogen concentration (Cocimano and Leng 1967; Nolan et al. 1970). McIntyre and Williams (1970) suggested that renal reabsorption of urea in sheep is related to the amount of urea presented to the kidney for filtration, and therefore depends on the circulating level of urea.

Thus, in general terms, the amount of nitrogen consumed tends to be directly related to the size of the ruminal ammonia pool which influences the rate of production of urea and therefore the concentration of urea in plasma (Nolan et al. 1970). Higher concentrations of plasma urea are associated with greater rates of urea recycling (i.e., rumen ammonia → plasma → rumen ammonia) by virtue of an increased concentration gradient between plasma and rumen fluid, but also result in larger losses of urea in the urine.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In contrast, the lower levels of plasma urea accompanying reduced nitrogen intakes result in decreased rates of urea recycling while enhancing a process of nitrogen conservation by presenting smaller amounts of urea to the kidney.

The results for reindeer are similar to those for sheep, in that the urinary excretion rate of urea reflects the concentration of urea in plasma. Thus, in reindeer as in sheep, reduced losses of nitrogen as urea may not be due to a special renal mechanism, but may be linked to the maintenance of the concentrations of urea in plasma at optimal levels. Whether the concentration of plasma urea is actively regulated in reindeer, or whether it fortuitously varies with nitrogen intake is a matter for further study.

The intake of snow and frozen feeds at low ambient temperatures may be significant from the standpoint of energy, as well as nitrogen balance. As discussed previously, the provision of liquid water at -5°C (trial D) was associated with a gain in body solids and an increase in nitrogen retention relative to the results of trial C (Figures 1 and 3). In the case of reindeer No. 9 the energy expenditure associated with consuming the respective quantities of snow and liquid water (values for water flux are used for convenience) can be calculated as approximately 700 and 200 kcal/day, or about 35 and 10% of the estimated rate of fasting katabolism, 2100 kcal/day ($70W^{3/4}$) (Kleiber 1961). Assuming that the apparent digestibility of organic matter is equivalent to that of energy, the apparently digestible energy intake for No. 9 may be estimated as 3600 kcal/day

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

during trials C and D. If the energy losses due to snow and water consumption are each subtracted from the latter figure, a daily surfeit of about 800 and 1300 kcal above the fasting metabolic requirement is calculated for trials C and D, respectively. Therefore, it appears that 800 kcal, less the presently unknown energy loss via urine and that due to the calorigenic effect of feed, was below the requirement for energy equilibrium. In contrast, of the 500 kcal/day (i.e., 1300-800 kcal/day) conserved as a result of the consumption of liquid water, a portion was available for production as indicated by the gain in body solids (Figure 1). Since the apparently digestible intake of nitrogen for No. 9 was similar in the two trials, the positive nitrogen balance observed in trial D (Figure 3) is attributable to decreased losses of urinary nitrogen. It appears that the increase in available energy associated with the consumption of liquid water somehow enhanced nitrogen retention.

Interpretation of Field Results

An attempt was made to calculate nitrogen intakes of grazing reindeer from the relationship established between water flux and nitrogen intake (Figure 2b). The daily loss of insensible water at the mean temperature associated with each field observation (Chap. 1, Table 1) was determined from the relationship between insensible water loss and ambient temperature (Figure 7), and the amount in excess of that lost at -20°C was subtracted from each seasonal estimate of water flux (Chap. 1, Table 2); the resultant values were applied to the regression in Figure 2b. Thus, in December 1968 and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

March 1969, the mean daily intakes of nitrogen were estimated to be 10 and 22 g, respectively. In December 1969 and March 1970 the respective estimates were 27 and 30 g nitrogen/day. As discussed in Chapter 1, it is thought that the high water flux rates obtained in May 1969 and June 1970 were due to the consumption of excessively wet forage; therefore, those values result in unrealistically high intakes of nitrogen and consequently will be omitted from consideration in the present discussion. Unfortunately, observations were not made during the period from late June to early August 1969 when maximum nitrogen intakes would be expected. Measurements made in late August 1969 coincide with estrus and the associated voluntary reductions in feed intake (McEwan 1968) are indicated by a calculated nitrogen intake of only 16 g/day. However, this estimate does not reflect the obvious improvement of body condition subsequent to the summer fattening period which is indicated by the increase in body solids between May and August 1969 (Chap. 1, Figure 1).

McEwan and Whitehead (1970) estimated the amount of apparently digestible nitrogen necessary for nitrogen equilibrium as 0.462 g/day/$kg^{3/4}$ for both reindeer and caribou. This value was obtained by feeding various amounts of a single ration which contained 21% crude protein and by determining the intake of apparently digestible nitrogen which corresponded to zero nitrogen balance. If the results of the pellet-feeding trials of the present study are similarly treated, the nitrogen requirement is estimated at 0.8 g/day/$kg^{3/4}$. It is difficult to resolve this descrepancy but a possible explanation is

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the differing protein-energy ratios of the ration used by McEwan and Whitehead (1970) and that consumed by the reindeer in this study.

McEwan and Whitehead obtained a mean of 73% for the apparent digestibility of nitrogen, and this value was applied to the above approximations for the nitrogen intakes of grazing reindeer. Thus, nitrogen intakes for December 1968 and March 1969 are calculated as 0.272 and 0.569 g day/$kg^{3/4}$, respectively; and for December 1969 and March 1970 as 0.614 and 0.704 of nitrogen/day/$kg^{3/4}$, respectively. Relative to that reported by McEwan and Whitehead the estimate in December 1968 is far below, while those in March 1969, December 1969 and March 1970 are in excess of, the nitrogen requirement. The body composition data for March and December 1969 do not support this conclusion, as the reindeer in both of these instances were lower in body solids than in the trial immediately preceding each observation (Chap. 1, Figure 1). However, the estimated mean of 0.704 g nitrogen/ day/$kg^{3/4}$ for March 1970 corresponds with a maintenance of body solids relative to the observation made in the previous December (Chap. 1, Figure 1). Hence, the present data suggest that the required intake of apparently digestible nitrogen for maintenance is between 0.7 and 0.8 g/day/$kg^{3/4}$. Further, the results of nitrogen balance (Figure 3) indicate that the pelleted ration consumed by the reindeer in the present study was of marginal nitrogen content, and suggest that a crude protein content in excess of 13% is required under most circumstances for maintenance of nitrogen equilibrium.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Criticism of the Experiment

Any interpretation of results involving only two animals must be made with caution and in full consideration of factors which might bias such an interpretation. The reindeer used in this study were appreciably different in body weight; although this may have resulted in subtle differences in response to the various treatments, no consistent variations were noted with respect to either plasma parameters or balance data (Figure 3).

The various conditions chosen for this study were intended to simulate the seasonality of feed intake, feed quality and temperature to which grazing reindeer are exposed. There are obvious differences in the metabolic responses of grazing animals as compared with those held in confinement, not only with regard to the daily procurement of food, but also those associated with behavioral patterns. The effects of restraint per se may have further altered the results from the normal pattern. Knox et al. (1969) found that mule deer closely confined in metabolism stalls had significantly higher rates of water flux compared with those held in a large room, while in contrast, water flux of stanchioned cattle reportedly decreases by 30 - 50% relative to that of cattle given the relative freedom of small corral (Longhurst et al. 1970). Thus, the reports are conflicting and it is not possible to evaluate the effects of confinement in reindeer until the appropriate comparative studies have been documented.

The determination of nitrogen, sodium, and potassium balance are subject to procedural errors which should be mentioned. The various

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

concentrations in urine were based on samples which had been pooled in proportion to daily output, but since the 24 h weights of feces varied within a much narrower range for each trial, the various concentrations were determined from fecal samples which had been bulked on the basis of equal weight. Thus, only a small deviation from a truly representative concentration would result in a comparatively large error in daily output.

The daily production of metabolic water, as calculated here, is also subject to an error of unknown degree. Water produced as a result of protein katabolism was estimated from urinary nitrogen output and is probably a good approximation, but that due to the breakdown of fat and carbohydrate was based on their respective apparent digestibilities. This assumes that the amount of each absorbed from the alimentary tract represented the daily quantity oxidized, and would result in an overestimate of metabolic water production in a fattening animal, and probably an underestimate in the case of undernutrition when body substance is being utilized. However, such deviations were presumed to be of minor importance since even at the lowest rate of water turnover recorded, a 10% error in the determination of metabolic water production would result in only about a 3% error in the estimate of water input (Figure 4).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

SYNOPSIS

Richmond et al. (1962) have established an interspecies correlation between water turnover and body weight. Water turnover was found to vary as the 0.8 power of body weight for all species examined (i.e., mouse, rat, rabbit, dog, man, horse) except the kangaroo rat. This relationship is presented in Figure 1; additional points representing results for several ruminants, including reindeer, are shown for comparison. It is seen in Figure 1 that within a species the relationship between water flux and body weight may vary substantially with changes in season or nutritional treatment. In this regard, reindeer differ more than any species shown. The lowest water flux observed in the laboratory studies (Chapter 2) is far below that predicted by the interspecific relationship and the representative point deviates from the regression line nearly as much as that for the kangaroo rat; in contrast, the maximum water flux obtained for reindeer is far above that expected on the basis of body weight.

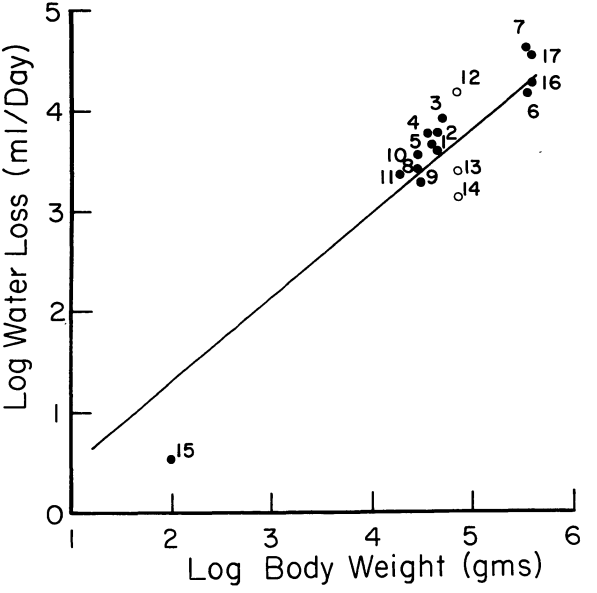
The intraspecific dependence of water flux on changes in ambient temperature and dietary regime points to a need for standardizing experimental conditions under which species are compared. Perhaps estimates of water flux should be made at maintenance, at a temperature common to the thermoneutral zone of all mammals, and with liquid water available free choice. The use of such "standard conditions" would permit not only a meaningful interspecific comparison, but would also provide a baseline for evaluating changes in water flux that occur

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

FIGURE 1. Interspecific correlation between log daily water loss and log body weight (Richmond et al. 1962) and its relation to reported results for other ruminants and the kangaroo rat.

Legend: 1. Sheep (stall-fed); Anand and Parker (1966)
2. " (pen-fed), winter; Longhurst et al. (1970)
3. " " , summer; " " " "
4. " (grazing), wet season; Macfarlane et al. (1966)
5. " " , dry " " " " "
6. Cattle (pen-fed); Macfarlane and Howard (1966)
7. " (grazing); " " " "
8. Mule deer (pen-fed); Knox et al. (1969)
9. Black-tailed deer (pen-fed), winter; Longhurst et al. (1970)
10. " " " " " , summer; Longhurst et al. (1970)
11. Antelope (pen-fed); Wesley et al. (1970)
12. Reindeer (grazing), spring-max. value obtained; Chap. 1, Table 2
" (stall-fed), +10°C-max. value obtained; Chap. 2, Table 3A
13. " (grazing), winter-min. value obtained; Chap. 1, Table 2
14. " (stall-fed), -20°C-min value obtained; Chap. 2, Table 3A
15. Kangaroo rat; Richmond et al. (1962)
16. Camel (grazing, hay supplement), winter; Siebert and Macfarlane (1971)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

within a species in response to other temperatures or nutritional treatments.

The camel is among the most drought-resistant of desert species, yet in winter, with adequate supplies of feed and water, water flux is near the value expected on the basis of body weight, and, as in other ruminants, increases at higher ambient temperatures. However, the camel differs from other ruminants in its response to water shortage. When deprived of water, camels rapidly limit urinary and fecal water output (Siebert and Macfarlane 1971) and as dehydration progresses, body temperature may rise as much as 3°C (Schmidt-Nielson, 1957b), thus reducing the need for evaporation of water as sweat. On the other hand, sheep lack the ability to rapidly reduce water losses, and consequently become dehydrated more quickly than camels, but can apparently tolerate a considerable degree of dehydration (Macfarlane et al. 1961).

The results of the present study do not permit an evaluation of the responses of reindeer to water shortage since the experiment was designed primarily to determine the degree to which various factors influence voluntary water intake. The results suggest however, that water losses are not under active regulation in this species. The excretion of fecal water appears to be directly related to fecal nitrogen losses (Chap. 2, Figure 5), and may account for the apparent dependence of water flux on the intake of nitrogen (Chap. 2, Figure 2b). Insensible water losses change as a function of temperature (Chap. 2, Figure 7) while urine volume is stoichiometrically related

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to water flux regardless of temperature or diet. Unlike camels, reindeer are extremely unresponsive to intravenous injections of vasopression (Macfarlane, personal communication), providing additional evidence that renal mechanisms are not in operation to control water losses.

McEwan (1968) reported that reindeer and caribou voluntarily reduce their feed intakes during winter; body weights are initially reduced and subsequently stabilize. Although this phenomenon is generally thought to result from changes in temperature and (or) photoperiod, one might speculate that the reduced appetite drive is related to the heat losses which would accompany the consumption of large quantities of snow. Thus, a reduced feed intake in winter may be a consequence of a "self-imposed" restriction of water intake.

It seems likely that, to a great extent, the successful adaptation of Rangifer to an arctic environment is related to the ability of the species to make metabolic adjustments in response to a state of undernutrition in order to minimize the utilization of body substance. Nitrogen may be the most critical nutrient to reindeer and caribou, and results of the present study suggest the existence of mechanisms by which urinary and fecal losses of nitrogen are minimized. Results of other work with reindeer in this laboratory (Luick et al. 1971) indicate an increase in the rate of glucose resynthesis in winter and spring when exogenous glucose precursors are in short supply. Since amino acids from mobilized body protein are important endogenous precursors of glucose, a mechanism for conserving glucose may be

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

associated with a reduced utilization of body protein. In addition, the nitrogen requirement of Rangifer may be lower in winter and spring than in summer, but such a possibility has not been investigated.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LITERATURE CITED

Anand, R. S. and Parker, H. R. 1966. Total body water and water turnover in sheep. Am. J. Vet. Res. 27:899-902.

Ashbacher, P. W., Kamal, T. H., and Cragle, R. G. 1965. Total body water estimations in dairy cattle using tritiated water. J. Animal Sci. 24:430-433.

Baker, N. F., Black, A. L., Anand, R. S., and Fisk, R. A. 1965. Body water turnover in cattle with parasitic gastroenteritis. Exp. Parasitol. 17:271-276.

Black, A. L., Baker, N. F., Bartley, J. C., Chapman, T. E., and Phillips, R. W. 1964. Water turnover in cattle. Science 144:876-878.

Bullard, R. W., Dill, D. B., and Yousef, M. K. 1970. Responses of the burro to desert heat stress. J. Appl. Physiol. 29:159-167.

Calder, F. W., Nicholson, J. W. G., and Cunningham, H. M. 1964. Water restriction for sheep on pasture and rate of consumption with other feeds. Can. J. Animal Sci. 44:266-271.

Cameron, R. D. and Luick, J. R. 1972. Seasonal changes in total body water, extracellular fluid, and blood volume in grazing reindeer. Can. J. Zool. 50:107-116.

Coady, J. W. and Gasaway, W. C. 1972. Rumen function and energy production of moose in interior Alaska. Eighth Ann. N. Amer. Moose Conf., Thunder Bay, Ontario.

Cocimano, M. R. and Leng, R. A. 1967. Metabolism of urea in sheep. Brit. J. Nutr. 21:353-371.

Consolazio, C. F., Johnson, R. E., and Pecora, L. J. 1963. Physiological measurements of metabolic functions in man. McGraw-Hill, Inc., New York.

Doornenbal, H. Asdell, S. A., and Comar, C. L. 1962. Relationship between Cr^{51}-determined total red cell volume and lean body mass in rats. J. Appl. Physiol. 17:737-740.

Farrell, D. 1970. Undernutrition in grazing sheep. Doctoral thesis, Dept. Biochemistry and Nutrition, University of New England, Armidale, N.S.W., Australia.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Forbes, J. M. 1968. The water intake of ewes. Brit. J. Nutr. 22:33-43.

Goodall, E. D. and Kay, R. N. B. 1968. Water intake and cycling of nitrogen to the stomach in sheep. J. Physiol. 194:38P-39P.

Harbin, R., Harbaugh, F. G., Neeley, K. L., and Fine, N. C. 1958. Effect of natural combinations of ambient temperature and relative humidity on the water intake of lactating and non-lactating dairy cows. J. Dairy Sci. 41:621-627.

Hecker, J. F., Budtz-Olsen, O. E., and Ostwald, M. 1964. The rumen as a water store in sheep. Aust. J. Agr. Res. 15:961-968.

Hodgetts, V. E. 1959. The influence of centrifugational treatment upon hematocrit values and the trapped plasma correction factor of sheep blood. Aust. J. Exp. Biol. Med. Sci. 37:97-106

Hodgetts, V. E. 1961. The dynamic red cell storage function of the spleen in sheep. III. Relationship to determination of blood volume, total red cell volume, and plasma volume. Aust. J. Exp. Biol. Med. Sci. 39:187-196.

Hoye, R. C. 1967. Simultaneous measurement of red cell, plasma, and extracellular fluid volume in the surgical patient. J. Lab. Clin. Med. 69:683-688.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York.

Jeffay, H., Olubajo, F. O., and Jewell, W. R. 1960. Determination of radioactive sulfur in biological materials. Anal. Chem. 32:306-308.

Johnson, H. D. and Yeck, R. G. 1964. Age and temperature effects on TDN, water consumption and balance of dairy calves and heifers exposed to environmental temperatures of 35 to 95°F. Mo. Agr. Exp. Sta. Res. Bull. 865.

Kamal, T. H. and Seif, S. M. 1969a. Changes in total body water and dry body weight with age and body weight in Friesians and water buffaloes. J. Dairy Sci. 52:1650-1656.

Kamal, T. H. and Seif, S. M. 1969b. Effect of natural and controlled climates of the Sahara on virtual tritium space in Friesians and water buffaloes. J. Dairy Sci. 52:1657-1663.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Kelsall, J. P. 1968. The Caribou. Dept. Indian Affairs and Northern Dev. Can. Wildlife Serv. Monogr. 3.

Kleiber, M. 1961. The Fire of Life. John Wiley and Sons, Inc., New York.

Klein, D. R. 1968. The introduction, increase, and crash of reindeer on St. Matthew Island. J. Wildlife Management. 32:350-367.

Klein, D. R. 1970. Tundra ranges north of the boreal forest. J. Range Management. 23:8-14.

Knox, K. L., Nagy, J. G., and Brown, R. D. 1969. Water turnover in mule deer. J. Wildlife Management. 33:389-393.

Livingston, H. G., Payne, W. J. A., and Friend, M. T. 1962. Urea excretion in ruminants. Nature (London) 194:1057-1058.

Longhurst, W. M., Baker, N. F., Connolly, G. E., and Fisk, R. A. 1970. Total body water and water turnover in sheep and deer. Amer. J. Vet. Res. 31:673-677.

Luick, J. R., Person, S. J., Cameron, R. D., and White, R. G. 1971. Seasonal variations in glucose metabolism of reindeer (Rangifer tarandus) using [U-^{14}C] and [3-^{3}H] glucose. Submitted to Brit. J. Nutr., March 1971.

Macfarlane, W. V., Morris, R. H. J., Howard, B., McDonald, J., and Budtz-Olsen, O. E. 1961. Water and electrolyte changes in tropical merino sheep exposed to dehydration during summer. Aust. J. Agr. Res. 12:889-912.

Macfarlane, W. V. 1964. Terrestrial animals in dry heat: ungulates. Handbook of Physiology, Sect. 4, Chap. 33, Waverly Press, Inc., Baltimore, Md.

Macfarlane, W. V., Morris, R. J. H., Howard, B. and Budtz-Olsen, O. E. 1959. Extracellular fluid distribution in tropical merino sheep. Aust. J. Agr. Res. 10:269-286.

Macfarlane, W. V., Dolling, C. H. S., and Howard, B. 1966. Distribution and turnover of water in merino sheep selected for high wool production. Aust. J. Agr. Res. 17:491-502.

Macfarlane, W. V. and Howard, B. 1966. Water content and turnover of identical twin Bos indicus and B. taurus in Kenya. J. Agr. Sci. 66:297-302.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Macfarlane, W. V., Howard, B., and Siebert, B. D. 1967. Water metabolism of merino and border leicester sheep grazing salt bush. Aust. J. Agr. Res. 18:947-958.

Maloiy, G. M. O. 1970. Water economy of the Somali donkey. Amer. J. Physiol. 219:1522-1527.

Maloiy, G. M. O., Kay, R. N. B., and Goodall, E. D. 1970. Digestion and nitrogen metabolism in sheep and red deer given large or small amounts of water and protein. Br. J. Nutr. 24:843-855.

Marsh, W. H., Fingerhut, B., and Miller, H. 1965. Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11:624-627.

McEwan, E. H. 1968. Growth and development of the barren-ground caribou. II. Postnatal growth rates. Can. J. Zool. 46:1023-1029.

McEwan, E. H. and Whitehead, P. E. 1970. Seasonal changes in the energy and nitrogen intake in reindeer and caribou. Can. J. Zool. 48:905-913.

McIntyre, K. H. 1970. The effects of increased nitrogen intakes on plasma urea nitrogen and rumen ammonia levels. Aust. J. Res. 21:501-507.

McIntyre, K. H. and Williams, V. J. 1970. The role of the kidney in nitrogen conservation in sheep. Aust. J. Exp. Biol. Med. Sci. 48:81-91.

Morris, R. H. J., Howard, B. and Macfarlane, W. V. 1962. Interaction of nutrition and air temperature with water metabolism of merino wethers shorn in winter. Aust. J. Agr. Res. 13:320-334.

Nolan, J. V., Cocimano, M. R., and Leng, R. A. 1970. Prediction of parameters of urea metabolism in sheep from the concentration of urea in plasma. Proc. Aust. Soc. Animal Prod. 8:22-28.

Pace, N. and Rathbun, E. N. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158:685-691.

Panaretto, B. A. 1963a. Body composition in vivo. III. The composition of living ruminants and its relation to the tritiated water spaces. Aust. J. Agr. Res. 14:944-952.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Panaretto, B. A. 1963b. The estimation of body composition in living animals. Symposium on carcase composition and appraisal of meat animals, Australia.

Panaretto, B. A. and Till, A. R. 1963. Body composition in vivo. II. The composition of mature goats and its relationship to antipyrine, tritiated water, and N-acetyl-4-aminoantipyrine spaces. Aust. J. Agr. Res. 14:926-943.

Panaretto, B. A. 1964. Body composition in vivo. VI. The composition of ewes during prolonged undernutrition. Aust. J. Agr. Res. 15:771-787.

Panaretto, B. A. and Little, D. A. 1965. Body composition in vivo. VII. The relation between red cell volume and total body water in ewes. Aust. J. Agr. Res. 16:661-665.

Phillips, R. W. and Knox, K. L. 1969. Water kinetics in enteric disease of neonatal calves. J. Dairy Sci. 52:1664-1668.

Pinson, E. A. 1952. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiol. Rev. 32:123-134.

Prentice, T. C., Siri, W., Berlin, N. I., Hyde, G. M., Parsons, R. J., Joiner, E. E., and Lawrence, J. H. 1952. Studies of total body water with tritium. J. Clin. Invest. 31:412-418.

Razdan, M. N., Sharma, D. D., Bhargava, P. K., and Chawla, M. S. 1971. Utilization of urea and water metabolism by Zebu cattle and buffaloes under tropical conditions. J. Dairy Sci. 54:1200-1206.

Richmond, C. R., Langham, W. H. and Trujillo, T. T. 1962. Comparative metabolism of tritiated water by mammals. J. Cell. Comp. Physiol. 59:45-53.

Rosenmann, M. and Morrison, P. 1967. Some effects of water deprivation in reindeer. Physiol. Zool. 40:134-142.

Schalm. O. W. 1965. Veterinary Hematology. 2nd ed. Lea and Febiger, Philadelphia, Pa.

Schmidt-Nielsen, K. 1964. Desert Animals. Oxford University Press, London.

Schmidt-Nielson, B., Schmidt-Nielson, D., Houpt, T. R., and Jarnum, S. A. 1957a. Urea excretion in the camel. Amer. J. Physiol. 188:477-484.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A., and Houpt, T. R. 1957b. Body temperature of the camel and its relation to water economy. Amer. J. Physiol. 188:103-122.

Searle, T. W. 1970. Body composition in lambs and young sheep and its prediction in vivo from tritiated water space and body weight. J. Agr. Sci., Camb. 74:357-362.

Searle, T. W. and Graham, N. McC. 1970. Body composition of growing sheep and its relevance to pasture evaluation. Proc. Aust. Soc. Animal Prod. 8:472-475.

Siebert, B. D. 1971. Growth and water metabolism of cows and progeny on fertilized and unfertilized tropical pastures. Aust. J. Agr. Res. 22:415-428.

Siebert, F. D. and Macfarlane, W. V. 1969. Body water content and water turnover of tropical Bos taurus, Bos indicus, Bibos banteng, and Bos bubalus bubalus. Aust. J. Agr. Res. 20:613-622.

Siebert, B. D. and Macfarlane, W. V. 1971. Water turnover and renal function of dromedaries in the desert. Physiol. Zool. 44:225-240.

Sperber, I. 1968. Phsyiological mechanisms in herbivours for retention and utilization of nitrogenous compounds. Isotope Studies on the Nitrogen Chain, International Atomic Energy Agency, Vienna.

Springell, P. H. 1968a. Water content and water turnover in beef cattle. Aust. J. Agr. Res. 19:129-144.

Springell, P. H. 1968b. Red cell volume and blood volume in beef cattle. Aust. J. Agr. Res. 19:-145-160.

Stahl, P. R. and Dale, H. E. 1958. Blood volumes of dairy calves comparing Cr^{51}-tagged red blood cells and T-1824 plasma dilution methods. Am. J. Physiol. 193:244-248.

Turner, A. W. and Hodgetts, V. E. 1959. The dynamic red cell storage function of the spleen in sheep. I. Relationship to fluctuations of jugular hematocrit. Aust. J. Exp. Biol. Med. Sci. 37:399-420.

Utley, P. R., Bradley, N. W., and Boling, J. A. 1970a. Effect of water restriction on nitrogen metabolism in bovine fed two levels of nitrogen. J. Nutr. 100:551-556.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Utley, P. R., Bradley, N. W., and Boling, J. A. 1970b. Effect of restricted water intake on feed intake, nutrient digestibility and nitrogen metabolism in steers. J. Animal Sci. 31:130-135.

Vercoe, J. E. 1967. Breed and nutritional effects on the composition of feces, urine, and plasma from Hereford and Brahman X Hereford steers fed on high and low quality diets. Aust. J. Agr. Res. 18:1003-1013.

Walser, M., Seldin, D. W. and Grollman, A. 1953. An evaluation of radiosulfate for the determination of the volume of extracellular fluid in man and dogs. J. Clin. Invest. 32:299-311.

Wesley, D. E. Knox, K. L., and Nagy, J. G. 1970. Energy flux and water kinetics in young pronghorn antelope. J. Wildlife Management. 34:908-912.

Weston, R. H. and Hogan, J. P. 1968. Rumen ammonia in relation to characteristics of the diet and parameters of nitrogen metabolism. Proc. Aust. Soc. Animal Prod. 7:359-363.

Wilson, A. D. 1966. The tolerance of sheep to sodium chloride in food or drinking water. Aust. J. Agr. Res. 17:503-514.

Winchester, C. F. and Morris, M. J. 1956. Water intake rates of cattle. J. Animal Sci. 15:722-740.

Yousef, M. K., Dill, D. B., and Mayes, M. G. 1970. Shifts in body fluids during dehydration in the burro, Equus asinus. J. Appl. Physiol. 29:345-349.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.